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(54) Title: MIRNA EXPRESSION IN HUMAN PERIPHERAL BLOOD MICROVESICLES AND USES THEREOF

(57) Abstract: The present invention provides novel methods and compositions for the diagnosis, prognosis and treatment of disorders by examining samples containing microvesicles and miRs therein.

## TITLE

**MIRNA EXPRESSION IN  
HUMAN PERIPHERAL BLOOD MICROVESICLES AND USES THEREOF**

Inventors: Clay B. Marsh, Melissa G. Hunter, Noura Ismail

**PRIORITY CLAIM AND  
STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH**

[0001] This application claims priority to U.S. Provisional Patent Application 60/993,809 filed September 14, 2007, and 61/055,178 filed May 22, 2008, which are fully incorporated herein by reference. This invention was not made with any government and the government has no rights in this invention.

**BACKGROUND OF THE INVENTION**

[0002] MicroRNAs (miRNAs or miRs) are small non-coding RNAs expressed in animals and plants. They regulate cellular function, cell survival, cell activation and cell differentiation during development.<sup>7,8</sup>

[0003] MicroRNAs are a small non-coding family of 19-25 nucleotide RNAs that regulate gene expression by targeting messenger RNAs (mRNA) in a sequence specific manner, inducing translational repression or mRNA degradation depending on the degree of complementarity between miRNAs and their targets (Bartel, D.P. (2004) *Cell* 116, 281-297; Ambros, V. (2004) *Nature* 431, 350-355). Many miRs are conserved in sequence between distantly related organisms, suggesting that these molecules participate in essential processes. Indeed, miRs are involved in the regulation of gene expression during development (Xu, P., *et al.* (2003) *Curr. Biol.* 13, 790-795), cell proliferation (Xu, P., *et al.* (2003) *Curr. Biol.* 13, 790-795), apoptosis (Cheng, A.M., *et al.* (2005) *Nucl. Acids Res.* 33, 1290-1297), glucose metabolism (Poy, M.N., *et al.* (2004) *Nature* 432, 226-230), stress resistance (Dresios, J., *et al.* (2005) *Proc. Natl. Acad. Sci. USA* 102, 1865-1870) and cancer (Calin, G.A., *et al.* (2002) *Proc. Natl. Acad. Sci. USA* 99, 1554-15529; Calin, G.A., *et al.* (2004) *Proc. Natl. Acad. Sci. USA* 101, 11755-11760; He, L., *et al.* (2005) *Nature* 435, 828-833; and Lu, J., *et al.* (2005) *Nature* 435:834-838).

[0004] There is also strong evidence that miRs play a role in mammalian hematopoiesis. In mice, miR-181, miR-223 and miR-142 are differentially expressed in

hematopoietic tissues, and their expression is regulated during hematopoiesis and lineage commitment (Chen, C.Z., *et al.* (2004) *Science* 303, 83-86). The ectopic expression of miR-181 in murine hematopoietic progenitor cells led to proliferation in the B-cell compartment (Chen, C.Z., *et al.* (2004) *Science* 303, 83-86). Systematic miR gene profiling in cells of the murine hematopoietic system revealed different miR expression patterns in the hematopoietic system compared with neuronal tissues, and identified individual miR expression changes that occur during cell differentiation (Monticelli, S., *et al.* (2005) *Genome Biology* 6, R71). A recent study has identified down-modulation of miR-221 and miR-222 in human erythropoietic cultures of CD34<sup>+</sup> cord blood progenitor cells (Felli, N., *et al.* (2005) *Proc. Natl. Acad. Sci. USA* 102, 18081-18086). These miRs were found to target the oncogene c-Kit. Further functional studies indicated that the decline of these two miRs in erythropoietic cultures unblocks Kit protein production at the translational level leading to expansion of early erythroid cells (Felli, N., *et al.* (2005) *Proc. Natl. Acad. Sci. USA* 102, 18081-18086). In line with the hypothesis of miRs regulating cell differentiation, miR-223 was found to be a key member of a regulatory circuit involving C/EBPa and NFI-A, which controls granulocytic differentiation in *all-trans* retinoic acid-treated acute promyelocytic leukemic cell lines (Fazi, F., *et al.* (2005) *Cell* 123, 819-831).

[0005] A frequent deletion and reduced expression of two miRs in B-cell chronic lymphocytic leukemia has been identified<sup>9</sup>. This discovery stimulated numerous articles documenting aberrant expression of miRs in head and neck carcinomas, small cell lung cancers, glioblastomas, breast cancers, chronic lymphocytic leukemia, and Burkitt lymphoma.<sup>9-12</sup> More recently, a relationship between inflammation and miRs has been reported in macrophages.<sup>13</sup>

[0006] In order to test for such disorders, tissue samples have been obtained in order to confirm the presence of such macrophages. In addition, until now, there has been no report demonstrating that microvesicles that circulate in the blood contain miRs.

[0007] Additional advantages, objects, and features of the invention will be set forth in part in the description which follows and in part will become apparent to those having ordinary skill in the art upon examination of the following or may be learned from practice of the invention. The objects and advantages of the invention may be realized and attained as particularly pointed out in the appended claims.

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## SUMMARY OF THE INVENTION

[0007A] In a first aspect, the present invention provides a method of diagnosing or prognosticating prostate cancer in a subject, comprising: i) isolating microvesicles from a peripheral blood sample of the subject; ii) determining the level of at least one miR gene product in the isolated microvesicles; and iii) comparing the level of the at least one miR gene product in the sample to a control, wherein an increase in the level of the at least one miR gene product in the sample from the subject, relative to that of the control, is diagnostic or prognostic of the prostate cancer; wherein the level of miR-21 and at least one of the following MiRs is determined: miR-15a, miR-16-1, miR-143 and miR-145.

[0007B] In a second aspect, the present invention provides use of isolated microvesicles as a biomarker for prostate cancer, wherein the biomarker is isolated from microvesicles in peripheral blood of a subject having the prostate cancer, and at least the following MiRs are upregulated in the isolated microvesicle relative to that of the control subject: miR-21, and at least the following miRs are down regulated in the isolated microvesicle relative to that of the control subject: miR-15a, miR-16-1, miR-143 and miR-145.

[0008] Described herein is a method for identifying specific miRs that are present in microvesicles and/or have altered expression levels of specific miRs in tissue, fluids and/or cells.

[0009] Microvesicles facilitate communication between cells. Many cells including macrophages, platelets, T -cells, and tumors release small microvesicles containing nucleic acids and/or proteins<sup>1-5</sup>. Factors contained within the microvesicles regulate angiogenesis, cell growth, and cell differentiation<sup>1-3</sup>.

[0010] As described herein, the presence of miRs in such fluids as peripheral blood of patients suffering from particular disorders is determined.

[0011] As described herein, the presence of miRs in lung tissue of patients suffering from pulmonary fibrosis is determined.

[0012] Described herein is a method of diagnosing or prognosticating a particular disorder in a subject (e.g., a human). According to one particular method, the level of at least one miR gene product in a test sample from the subject is compared to the level of a corresponding miR gene product in a control sample.

An alteration (e.g., an increase, a decrease) in the level of the miR gene product in the test sample, relative to the level of a corresponding miR gene product in the control sample, is

indicative of the subject either having, or being at risk for developing, an acute inflammatory disorder.

[0013] In one embodiment, the level of the miR gene product in the test sample from the subject is greater than that of the control. In another embodiment, the at least one miR gene product is selected from the group consisting of the miRNAs as shown herein.

[0014] In particular embodiments, the disorder that is diagnosed or prognosticated is one that causes mononuclear phagocytes and/or THP-1 cells to release microvesicles.

[0015] In particular embodiments, the disorder that is diagnosed or prognosticated is one that causes an inflammatory response.

[0016] In another embodiment, the invention is a method of treating a cancer and/or an inflammatory disorder in a subject (e.g., a human).

[0017] In one particular method, an effective amount of a compound for inhibiting expression of at least one miR gene product selected from the one or more of the groups found in Table I-VI is administered to the subject.

[0018] In one embodiment, the compound for inhibiting expression of at least one miR

gene product inhibits expression of a miR gene product selected from one or more of the groups found in Tables I-VI.

[0019] The invention further provides pharmaceutical compositions for treating cancer and/or an inflammatory disorder. In one embodiment, the pharmaceutical compositions of the invention comprise at least one miR expression-inhibition compound and a pharmaceutically-acceptable carrier. In a particular embodiment, the at least one miR expression-inhibition compound is specific for a miR gene product whose expression is greater in blood from diseased patients compared to normals.

[0020] In yet another embodiment, the pharmaceutical composition further comprises at least one anti-inflammatory agent.

[0021] In one embodiment, the invention is a pharmaceutical composition for treating a cancer associated with overexpression of a miR gene product and/or a lung disorder associated with overexpression of a miR gene product. Such pharmaceutical compositions comprise an effective amount of at least one miR gene product and a pharmaceutically-acceptable carrier, wherein the at least one miR gene product binds to, and decreases expression of, the miR gene product. In another embodiment, the at least one miR gene product comprises a nucleotide sequence that is complementary to a nucleotide sequence in the miR- gene product. In still another embodiment, the at least one miR gene product is miR- or a variant or biologically-active fragment thereof. In yet another embodiment, the pharmaceutical composition further comprises at least one anti-cancer agent.

[0022] Various objects and advantages of this invention will become apparent to those skilled in the art from the following detailed description of the preferred embodiment, when read in light of the accompanying drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **Figure 1** shows the differentiation induced release of microvesicles from macrophages. Peripheral blood monocytes (PBM) were untreated (light) or treated with GM-CSF (dark) for 24 h. Cell-free supernatant was collected and ultracentrifuged. The vesicles were resuspended in PBS and analyzed for size on a flow cytometry. Prior to analysis, FSS and SSC parameters were adjusted using 2  $\mu$ m standard beads (not shown). Shown is representative data from three different donors.

[0024] **Figures 2A-2C** show microvesicles mediate macrophage differentiation. Microvesicles were collected from PMA-treated THP1 cells then added to undifferentiated

THP1 cells (**Figure 2B**) or monocytes (**Figure 2C**). As a control, THP1 cells were left untreated (**Figure 2A**). The cells were photographed daily. Shown are the cells at day 3.

[0025] **Figures 3A-3C** show the isolation of peripheral blood microvesicles.

Following informed consent, plasma was obtained from 20cc of blood from normal volunteer donors. The microvesicles from 0.5 cc of plasma were incubated with CD206-FITC or MHCII-FITC antibodies and analyzed on BD FACS Calibur for size using forward vs. side scatter (**Figure 3A**) and surface antigen expression (**Figure 3B**). The percent expression of either CD206 or MHC II compared to isotype control was determined for the gated region shown in **Figure 3A** (**Figure 3C**). Shown is the average ± SEM of two donors.

[0026] **Figure 4. Analysis of the origin of peripheral blood microvesicles.**

Peripheral blood microvesicles from healthy donors (n=10) were analyzed by flow cytometry. To determine cell origin, microvesicles were stained for CD3, CD202b (Tie-2), CD66b, CD79a, or CD41a to determine those that originated from T-cells, endothelial cells, neutrophils, B-cells, or platelets. Mononuclear phagocyte-derived microvesicles were positive for CD14, CD206, CCR3, CCR2, or CCR5. Shown is the average % maximum of total gated events ± S.E.M.

[0027] **Figures 5A - 5D. miRNA expression from peripheral blood**

**microvesicles and PBMC. (Figure 5A)** Hierarchical cluster analysis for microvesicles and PBMC is shown based on filtering criteria. Heat-maps demonstrating the expression profile for microvesicles (**Figure 5B**) and PBMC (**Figure 5C**) were generated. (**Figure 5D**). The number of shared and specific for each sample group is shown.

[0028] **Figure 6: Table I** showing various diseases and up- and down-regulated miRs associated therewith. microRNAs that are important in tissue of human diseases, including cancer and non-cancer applications are listed. Comparing miRNAs that are undetectable in the plasma from our data set (**Figure 7, Table II**) with miRNAs known to increase in the tissue of specific diseases, the inventors now believe that we predict that several miRNAs may serve as biomarkers in the plasma (see miRs in **bold** in **Figure 6**, Table I Increase Expression Column).

[0029] **Figure 7: Table II** showing miRs that are expressed in the plasma and those that are undetectable.

[0030] **Figure 8: Table III** lists miRs and show the top ten expressed miRNAs in the plasma microvesicles and the PBMC from all individuals.

[0031] **Figure 9: Table IV** showing canonical pathways involved in metabolism and regulation of the acquired immune system were highly regulated by the expression of these miRNAs using Sanger miRBase alone (top) or common targets from Sanger miRBase and TargetScan (bottom).

[0032] **Figure 10: Table V** showing that 20 miRNAs had more than a three-fold increase in expression in the PBMC fraction compared to the microvesicles plasma samples as well as the fold change in plasma microvesicles compared to PMBC (last Column)

[0033] **Figure 11: Table VI** showing, the normalized expression data for all detected miRs: detector name, ave -MNC, std-MNC, detector name, ave-serum, std-serum.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0034] The present invention is based, in part, on the identification of specific microRNAs (miRNAs) that are involved in an inflammatory response and/or have altered expression levels in blood. The invention is further based, in part, on association of these miRNAs with particular diagnostic, prognostic and therapeutic features.

[0035] As described and exemplified herein particular miRNA are up- or down-regulated during tissue injury and/or inflammation.

[0036] As used herein interchangeably, a "miR gene product," "microRNA," "miR," "miR" or "miRNA" refers to the unprocessed or processed RNA transcript from a miR gene. As the miR gene products are not translated into protein, the term "miR gene products" does not include proteins. The unprocessed miR gene transcript is also called a "miR precursor," and typically comprises an RNA transcript of about 70-100 nucleotides in length. The miR precursor can be processed by digestion with an RNase (for example, Dicer, Argonaut, RNase III (e.g., *E. coli* RNase III)) into an active 19-25 nucleotide RNA molecule. This active 19-25 nucleotide RNA molecule is also called the "processed" miR gene transcript or "mature" miRNA.

[0037] The active 19-25 nucleotide RNA molecule can be obtained from the miR precursor through natural processing routes (e.g., using intact cells or cell lysates) or by synthetic processing routes (e.g., using isolated processing enzymes, such as isolated Dicer, Argonaut, or RNase III). It is understood that the active 19-25 nucleotide RNA molecule can also be produced directly by biological or chemical synthesis, without having to be processed from the miR precursor. When a microRNA is referred to herein

by name, the name corresponds to both the precursor and mature forms, unless otherwise indicated.

[0038] The present invention encompasses methods of diagnosing or prognosticating whether a subject has, or is at risk for developing, a disorder where microvesicles are released.

[0039] The methods comprise determining the level of at least one miR gene product in a sample from the subject and comparing the level of the miR gene product in the sample to a control. As used herein, a "subject" can be any mammal that has, or is suspected of having, such disorder. In a preferred embodiment, the subject is a human who has, or is suspected of having, such disorder.

[0040] The level of at least one miR gene product can be measured in cells of a biological sample obtained from the subject.

[0041] In another embodiment, a sample can be removed from the subject, and DNA can be extracted and isolated by standard techniques. For example, in certain embodiments, the sample can be obtained from the subject prior to initiation of radiotherapy, chemotherapy or other therapeutic treatment. A corresponding control sample, or a control reference sample (e.g., obtained from a population of control samples), can be obtained from unaffected samples of the subject, from a normal human individual or population of normal individuals, or from cultured cells corresponding to the majority of cells in the subject's sample. The control sample can then be processed along with the sample from the subject, so that the levels of miR gene product produced from a given miR gene in cells from the subject's sample can be compared to the corresponding miR gene product levels from cells of the control sample. Alternatively, a reference sample can be obtained and processed separately (e.g., at a different time) from the test sample and the level of a miR gene product produced from a given miR gene in cells from the test sample can be compared to the corresponding miR gene product level from the reference sample.

[0042] In one embodiment, the level of the at least one miR gene product in the test sample is greater than the level of the corresponding miR gene product in the control sample (i.e., expression of the miR gene product is "upregulated"). As used herein, expression of a miR gene product is "upregulated" when the amount of miR gene product in a sample from a subject is greater than the amount of the same gene product in a control (for example, a reference standard, a control cell sample, a control tissue sample).

[0043] In another embodiment, the level of the at least one miR gene product in the test sample is less than the level of the corresponding miR gene product in the control sample (i.e., expression of the miR gene product is "downregulated"). As used herein, expression of a miR gene is "downregulated" when the amount of miR gene product produced from that gene in a sample from a subject is less than the amount produced from the same gene in a control sample. The relative miR gene expression in the control and normal samples can be determined with respect to one or more RNA expression standards. The standards can comprise, for example, a zero miR gene expression level, the miR gene expression level in a standard cell line, the miR gene expression level in unaffected samples of the subject, or the average level of miR gene expression previously obtained for a population of normal human controls (e.g., a control reference standard).

[0044] The level of the at least one miR gene product can be measured using a variety of techniques that are well known to those of skill in the art (e.g., quantitative or semi-quantitative RT-PCR, Northern blot analysis, solution hybridization detection). In a particular embodiment, the level of at least one miR gene product is measured by reverse transcribing RNA from a test sample obtained from the subject to provide a set of target oligodeoxynucleotides, hybridizing the target oligodeoxynucleotides to one or more miRNA-specific probe oligonucleotides (e.g., a microarray that comprises miRNA-specific probe oligonucleotides) to provide a hybridization profile for the test sample, and comparing the test sample hybridization profile to a hybridization profile generated from a control sample. An alteration in the signal of at least one miRNA in the test sample relative to the control sample is indicative of the subject either having, or being at risk for a particular disorder.

[0045] Also, a microarray can be prepared from gene-specific oligonucleotide probes generated from known miRNA sequences. The array may contain two different oligonucleotide probes for each miRNA, one containing the active, mature sequence and the other being specific for the precursor of the miRNA. The array may also contain controls, such as one or more mouse sequences differing from human orthologs by only a few bases, which can serve as controls for hybridization stringency conditions. tRNAs and other RNAs (e.g., rRNAs, mRNAs) from both species may also be printed on the microchip, providing an internal, relatively stable, positive control for specific hybridization. One or more appropriate controls for non-specific hybridization may also be included on the microchip. For this purpose, sequences are selected based upon the

absence of any homology with any known miRNAs.

[0046] The microarray may be fabricated using techniques known in the art. For example, probe oligonucleotides of an appropriate length, e.g., 40 nucleotides, are 5'-amine modified at position C6 and printed using commercially available microarray systems, e.g., the GeneMachine OmniGrid™ 100 Microarrayer and Amersham CodeLink™ activated slides. Labeled cDNA oligomer corresponding to the target RNAs is prepared by reverse transcribing the target RNA with labeled primer. Following first strand synthesis, the RNA/DNA hybrids are denatured to degrade the RNA templates. The labeled target cDNAs thus prepared are then hybridized to the microarray chip under hybridizing conditions, e.g., 6X SSPE/30% formamide at 25°C for 18 hours, followed by washing in 0.75X TNT at 37°C for 40 minutes. At positions on the array where the immobilized probe DNA recognizes a complementary target cDNA in the sample, hybridization occurs. The labeled target cDNA marks the exact position on the array where binding occurs, allowing automatic detection and quantification. The output consists of a list of hybridization events, indicating the relative abundance of specific cDNA sequences, and therefore the relative abundance of the corresponding complementary miRs, in the patient sample. According to one embodiment, the labeled cDNA oligomer is a biotin-labeled cDNA, prepared from a biotin-labeled primer. The microarray is then processed by direct detection of the biotin-containing transcripts using, e.g., Streptavidin-Alexa647 conjugate, and scanned utilizing conventional scanning methods. Image intensities of each spot on the array are proportional to the abundance of the corresponding miR in the patient sample.

[0047] The use of the array has several advantages for miRNA expression detection. First, the global expression of several hundred genes can be identified in the same sample at one time point. Second, through careful design of the oligonucleotide probes, expression of both mature and precursor molecules can be identified. Third, in comparison with Northern blot analysis, the chip requires a small amount of RNA, and provides reproducible results using 2.5 µg of total RNA. The relatively limited number of miRNAs (a few hundred per species) allows the construction of a common microarray for several species, with distinct oligonucleotide probes for each. Such a tool allows for analysis of trans-species expression for each known miR under various conditions.

[0048] In addition to use for quantitative expression level assays of specific miRs, a microchip containing miRNA-specific probe oligonucleotides corresponding to a

substantial portion of the miRNome, preferably the entire miRNome, may be employed to carry out miR gene expression profiling, for analysis of miR expression patterns. Distinct miR signatures can be associated with established disease markers, or directly with a disease state.

[0049] According to the expression profiling methods described herein, total RNA from a sample from a subject suspected of having a particular disorder is quantitatively reverse transcribed to provide a set of labeled target oligodeoxynucleotides complementary to the RNA in the sample. The target oligodeoxynucleotides are then hybridized to a microarray comprising miRNA-specific probe oligonucleotides to provide a hybridization profile for the sample. The result is a hybridization profile for the sample representing the expression pattern of miRNA in the sample. The hybridization profile comprises the signal from the binding of the target oligodeoxynucleotides from the sample to the miRNA-specific probe oligonucleotides in the microarray. The profile may be recorded as the presence or absence of binding (signal vs. zero signal). More preferably, the profile recorded includes the intensity of the signal from each hybridization. The profile is compared to the hybridization profile generated from a normal control sample or reference sample. An alteration in the signal is indicative of the presence of, or propensity to develop, the particular disorder in the subject.

[0050] Other techniques for measuring miR gene expression are also within the skill in the art, and include various techniques for measuring rates of RNA transcription and degradation.

[0051] The invention also provides methods of diagnosing whether a subject has, or is at risk for developing, a particular disorder with an adverse prognosis. In this method, the level of at least one miR gene product, which is associated with an adverse prognosis in a particular disorder, is measured by reverse transcribing RNA from a test sample obtained from the subject to provide a set of target oligodeoxynucleotides. The target oligodeoxynucleotides are then hybridized to one or more miRNA-specific probe oligonucleotides (e.g., a microarray that comprises miRNA-specific probe oligonucleotides) to provide a hybridization profile for the test sample, and the test sample hybridization profile is compared to a hybridization profile generated from a control sample. An alteration in the signal of at least one miRNA in the test sample relative to the control sample is indicative of the subject either having, or being at risk for developing, a particular disorder with an adverse prognosis.

[0052] In some instances, it may be desirable to simultaneously determine the expression level of a plurality of different miR gene products in a sample. In other instances, it may be desirable to determine the expression level of the transcripts of all known miR genes correlated with a particular disorder. Assessing specific expression levels for hundreds of miR genes or gene products is time consuming and requires a large amount of total RNA (e.g., at least 20 µg for each Northern blot) and autoradiographic techniques that require radioactive isotopes.

[0053] To overcome these limitations, an oligolibrary, in microchip format (i.e., a microarray), may be constructed containing a set of oligonucleotide (e.g., oligodeoxynucleotide) probes that are specific for a set of miR genes. Using such a microarray, the expression level of multiple microRNAs in a biological sample can be determined by reverse transcribing the RNAs to generate a set of target oligodeoxynucleotides, and hybridizing them to probe the oligonucleotides on the microarray to generate a hybridization, or expression, profile. The hybridization profile of the test sample can then be compared to that of a control sample to determine which microRNAs have an altered expression level. As used herein, "probe oligonucleotide" or "probe oligodeoxynucleotide" refers to an oligonucleotide that is capable of hybridizing to a target oligonucleotide. "Target oligonucleotide" or "target oligodeoxynucleotide" refers to a molecule to be detected (e.g., via hybridization). By "miR-specific probe oligonucleotide" or "probe oligonucleotide specific for a miR" is meant a probe oligonucleotide that has a sequence selected to hybridize to a specific miR gene product, or to a reverse transcript of the specific miR gene product.

[0054] An "expression profile" or "hybridization profile" of a particular sample is essentially a fingerprint of the state of the sample; while two states may have any particular gene similarly expressed, the evaluation of a number of genes simultaneously allows the generation of a gene expression profile that is unique to the state of the cell. That is, normal samples may be distinguished from corresponding disorder-exhibiting samples. Within such disorder-exhibiting samples, different prognosis states (for example, good or poor long term survival prospects) may be determined. By comparing expression profiles of disorder-exhibiting samples in different states, information regarding which genes are important (including both upregulation and downregulation of genes) in each of these states is obtained.

[0055] The identification of sequences that are differentially expressed in disorder-

exhibiting samples, as well as differential expression resulting in different prognostic outcomes, allows the use of this information in a number of ways. For example, a particular treatment regime may be evaluated (e.g., to determine whether a chemotherapeutic drug acts to improve the long-term prognosis in a particular subject). Similarly, diagnosis may be done or confirmed by comparing samples from a subject with known expression profiles. Furthermore, these gene expression profiles (or individual genes) allow screening of drug candidates that suppress the particular disorder expression profile or convert a poor prognosis profile to a better prognosis profile.

[0056] Alterations in the level of one or more miR gene products in cells can result in the deregulation of one or more intended targets for these miRs, which can lead to a particular disorder. Therefore, altering the level of the miR gene product (e.g., by decreasing the level of a miR that is upregulated in disorder-exhibiting cells, by increasing the level of a miR that is downregulated in disorder-exhibiting cells) may successfully treat the disorder.

[0057] Accordingly, the present invention encompasses methods of treating a disorder in a subject, wherein at least one miR gene product is deregulated (e.g., downregulated, upregulated) in the cells of the subject. In one embodiment, the level of at least one miR gene product in a test sample is greater than the level of the corresponding miR gene product in a control or reference sample. In another embodiment, the level of at least one miR gene product in a test sample is less than the level of the corresponding miR gene product in a control sample. When the at least one isolated miR gene product is downregulated in the test sample, the method comprises administering an effective amount of the at least one isolated miR gene product, or an isolated variant or biologically-active fragment thereof, such that proliferation of the disorder-exhibiting cells in the subject is inhibited.

[0058] For example, when a miR gene product is downregulated in a cancer cell in a subject, administering an effective amount of an isolated miR gene product to the subject can inhibit proliferation of the cancer cell. The isolated miR gene product that is administered to the subject can be identical to an endogenous wild-type miR gene product that is downregulated in the cancer cell or it can be a variant or biologically-active fragment thereof.

[0059] As defined herein, a "variant" of a miR gene product refers to a miRNA that has less than 100% identity to a corresponding wild-type miR gene product and possesses

one or more biological activities of the corresponding wild-type miR gene product. Examples of such biological activities include, but are not limited to, inhibition of expression of a target RNA molecule (e.g., inhibiting translation of a target RNA molecule, modulating the stability of a target RNA molecule, inhibiting processing of a target RNA molecule) and inhibition of a cellular process associated with cancer and/or a myeloproliferative disorder (e.g., cell differentiation, cell growth, cell death). These variants include species variants and variants that are the consequence of one or more mutations (e.g., a substitution, a deletion, an insertion) in a miR gene. In certain embodiments, the variant is at least about 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to a corresponding wild-type miR gene product.

[0060] As defined herein, a "biologically-active fragment" of a miR gene product refers to an RNA fragment of a miR gene product that possesses one or more biological activities of a corresponding wild-type miR gene product. As described above, examples of such biological activities include, but are not limited to, inhibition of expression of a target RNA molecule and inhibition of a cellular process associated with cancer and/or a myeloproliferative disorder. In certain embodiments, the biologically-active fragment is at least about 5, 7, 10, 12, 15, or 17 nucleotides in length. In a particular embodiment, an isolated miR gene product can be administered to a subject in combination with one or more additional anti-cancer treatments. Suitable anti-cancer treatments include, but are not limited to, chemotherapy, radiation therapy and combinations thereof (e.g., chemoradiation).

[0061] When the at least one isolated miR gene product is upregulated in the cancer cells, the method comprises administering to the subject an effective amount of a compound that inhibits expression of the at least one miR gene product, such that proliferation of the disorder-exhibiting cells is inhibited. Such compounds are referred to herein as miR gene expression-inhibition compounds. Examples of suitable miR gene expression-inhibition compounds include, but are not limited to, those described herein (e.g., double-stranded RNA, antisense nucleic acids and enzymatic RNA molecules).

[0062] In a particular embodiment, a miR gene expression-inhibiting compound can be administered to a subject in combination with one or more additional anti-cancer treatments. Suitable anti-cancer treatments include, but are not limited to, chemotherapy, radiation therapy and combinations thereof (e.g., chemoradiation).

[0063] As described herein, when the at least one isolated miR gene product is

upregulated in cancer cells, the method comprises administering to the subject an effective amount of at least one compound for inhibiting expression of the at least one miR gene product, such that proliferation of cancer cells is inhibited.

[0064] The terms “treat”, “treating” and “treatment”, as used herein, refer to ameliorating symptoms associated with a disease or condition, for example, cancer and/or other condition or disorder, including preventing or delaying the onset of the disease symptoms, and/or lessening the severity or frequency of symptoms of the disease, disorder or condition. The terms “subject”, “patient” and “individual” are defined herein to include animals, such as mammals, including, but not limited to, primates, cows, sheep, goats, horses, dogs, cats, rabbits, guinea pigs, rats, mice or other bovine, ovine, equine, canine, feline, rodent, or murine species. In a preferred embodiment, the animal is a human.

[0065] As used herein, an “isolated” miR gene product is one that is synthesized, or altered or removed from the natural state through human intervention. For example, a synthetic miR gene product, or a miR gene product partially or completely separated from the coexisting materials of its natural state, is considered to be “isolated.” An isolated miR gene product can exist in a substantially-purified form, or can exist in a cell into which the miR gene product has been delivered. Thus, a miR gene product that is deliberately delivered to, or expressed in, a cell is considered an “isolated” miR gene product. A miR gene product produced inside a cell from a miR precursor molecule is also considered to be an “isolated” molecule. According to the invention, the isolated miR gene products described herein can be used for the manufacture of a medicament for treating a subject (e.g., a human).

[0066] Isolated miR gene products can be obtained using a number of standard techniques. For example, the miR gene products can be chemically synthesized or recombinantly produced using methods known in the art. In one embodiment, miR gene products are chemically synthesized using appropriately protected ribonucleoside phosphoramidites and a conventional DNA/RNA synthesizer. Commercial suppliers of synthetic RNA molecules or synthesis reagents include, e.g., Proligo (Hamburg, Germany), Dharmacon Research (Lafayette, CO, U.S.A.), Pierce Chemical (part of Perbio Science, Rockford, IL, U.S.A.), Glen Research (Sterling, VA, U.S.A.), ChemGenes (Ashland, MA, U.S.A.) and Cruachem (Glasgow, UK).

[0067] Alternatively, the miR gene products can be expressed from recombinant circular or linear DNA plasmids using any suitable promoter. Suitable promoters for

expressing RNA from a plasmid include, e.g., the U6 or H1 RNA pol III promoter sequences, or the cytomegalovirus promoters. Selection of other suitable promoters is within the skill in the art. The recombinant plasmids of the invention can also comprise inducible or regulatable promoters for expression of the miR gene products in cells (e.g., cancerous cells, cells exhibiting a myeloproliferative disorder).

[0068] The miR gene products that are expressed from recombinant plasmids can be isolated from cultured cell expression systems by standard techniques. The miR gene products that are expressed from recombinant plasmids can also be delivered to, and expressed directly in, cells.

[0069] The miR gene products can be expressed from a separate recombinant plasmid, or they can be expressed from the same recombinant plasmid. In one embodiment, the miR gene products are expressed as RNA precursor molecules from a single plasmid, and the precursor molecules are processed into the functional miR gene product by a suitable processing system, including, but not limited to, processing systems extant within a cancer cell.

[0070] Selection of plasmids suitable for expressing the miR gene products, methods for inserting nucleic acid sequences into the plasmid to express the gene products, and methods of delivering the recombinant plasmid to the cells of interest are within the skill in the art. See, for example, Zeng *et al.* (2002), *Molecular Cell* 9:1327-1333; Tuschl (2002), *Nat. Biotechnol.* 20:446-448; Brummelkamp *et al.* (2002), *Science* 296:550-553; Miyagishi *et al.* (2002), *Nat. Biotechnol.* 20:497-500; Paddison *et al.* (2002), *Genes Dev.* 16:948-958; Lee *et al.* (2002), *Nat. Biotechnol.* 20:500-505; and Paul *et al.* (2002), *Nat. Biotechnol.* 20:505-508, the entire disclosures of which are incorporated herein by reference. For example, in certain embodiments, a plasmid expressing the miR gene products can comprise a sequence encoding a miR precursor RNA under the control of the CMV intermediate-early promoter. As used herein, “under the control” of a promoter means that the nucleic acid sequences encoding the miR gene product are located 3' of the promoter, so that the promoter can initiate transcription of the miR gene product coding sequences.

[0071] The miR gene products can also be expressed from recombinant viral vectors. It is contemplated that the miR gene products can be expressed from two separate recombinant viral vectors, or from the same viral vector. The RNA expressed from the recombinant viral vectors can either be isolated from cultured cell expression systems by

standard techniques, or can be expressed directly in cells (e.g., cancerous cells, cells exhibiting a myeloproliferative disorder).

[0072] In other embodiments of the treatment methods of the invention, an effective amount of at least one compound that inhibits miR expression can be administered to the subject. As used herein, “inhibiting miR expression” means that the production of the precursor and/or active, mature form of miR gene product after treatment is less than the amount produced prior to treatment. One skilled in the art can readily determine whether miR expression has been inhibited in cells using, for example, the techniques for determining miR transcript level discussed herein. Inhibition can occur at the level of gene expression (i.e., by inhibiting transcription of a miR gene encoding the miR gene product) or at the level of processing (e.g., by inhibiting processing of a miR precursor into a mature, active miR).

[0073] As used herein, an “effective amount” of a compound that inhibits miR expression is an amount sufficient to inhibit proliferation of cells in a subject suffering from cancer and/or a myeloproliferative disorder. One skilled in the art can readily determine an effective amount of a miR expression-inhibiting compound to be administered to a given subject, by taking into account factors, such as the size and weight of the subject; the extent of disease penetration; the age, health and sex of the subject; the route of administration; and whether the administration is regional or systemic.

[0074] One skilled in the art can also readily determine an appropriate dosage regimen for administering a compound that inhibits miR expression to a given subject, as described herein. Suitable compounds for inhibiting miR gene expression include double-stranded RNA (such as short- or small-interfering RNA or “siRNA”), antisense nucleic acids, and enzymatic RNA molecules, such as ribozymes. Each of these compounds can be targeted to a given miR gene product and interfere with the expression (e.g., by inhibiting translation, by inducing cleavage and/or degradation) of the target miR gene product.

[0075] For example, expression of a given miR gene can be inhibited by inducing RNA interference of the miR gene with an isolated double-stranded RNA (“dsRNA”) molecule which has at least 90%, for example, at least 95%, at least 98%, at least 99%, or 100%, sequence homology with at least a portion of the miR gene product. In a particular embodiment, the dsRNA molecule is a “short or small interfering RNA” or “siRNA.”

[0076] Administration of at least one miR gene product, or at least one compound for inhibiting miR expression, will inhibit the proliferation of cells (e.g., cancerous cells, cells

exhibiting a myeloproliferative disorder) in a subject who has a cancer and/or a myeloproliferative disorder. As used herein, to “inhibit the proliferation of cancerous cells or cells exhibiting a myeloproliferative disorder” means to kill the cells, or permanently or temporarily arrest or slow the growth of the cells. Inhibition of cell proliferation can be inferred if the number of such cells in the subject remains constant or decreases after administration of the miR gene products or miR gene expression-inhibiting compounds. An inhibition of proliferation of cancerous cells or cells exhibiting a myeloproliferative disorder can also be inferred if the absolute number of such cells increases, but the rate of tumor growth decreases.

[0077] A miR gene product or miR gene expression-inhibiting compound can also be administered to a subject by any suitable enteral or parenteral administration route.

Suitable enteral administration routes for the present methods include, e.g., oral, rectal, or intranasal delivery. Suitable parenteral administration routes include, e.g., intravascular administration (e.g., intravenous bolus injection, intravenous infusion, intra-arterial bolus injection, intra-arterial infusion and catheter instillation into the vasculature); peri- and intra-tissue injection (e.g., peri-tumoral and intra-tumoral injection, intra-retinal injection, or subretinal injection); subcutaneous injection or deposition, including subcutaneous infusion (such as by osmotic pumps); direct application to the tissue of interest, for example by a catheter or other placement device (e.g., a retinal pellet or a suppository or an implant comprising a porous, non-porous, or gelatinous material); and inhalation. Particularly suitable administration routes are injection, infusion and direct injection into the tumor.

[0078] The miR gene products or miR gene expression-inhibition compounds can be formulated as pharmaceutical compositions, sometimes called “medicaments,” prior to administering them to a subject, according to techniques known in the art. Accordingly, the invention encompasses pharmaceutical compositions for treating cancer and/or a myeloproliferative disorder.

[0079] The present pharmaceutical compositions comprise at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising a sequence encoding the miR gene product or miR gene expression-inhibition compound) (e.g., 0.1 to 90% by weight), or a physiologically-acceptable salt thereof, mixed with a pharmaceutically-acceptable carrier. In certain embodiments, the pharmaceutical composition of the invention additionally comprises one or more anti-

cancer agents (e.g., chemotherapeutic agents). The pharmaceutical formulations of the invention can also comprise at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising a sequence encoding the miR gene product or miR gene expression-inhibition compound), which are encapsulated by liposomes and a pharmaceutically-acceptable carrier.

[0080] Pharmaceutical compositions of the invention can also comprise conventional pharmaceutical excipients and/or additives. Suitable pharmaceutical excipients include stabilizers, antioxidants, osmolality adjusting agents, buffers, and pH adjusting agents. Suitable additives include, e.g., physiologically biocompatible buffers (e.g., tromethamine hydrochloride), additions of chelants (such as, for example, DTPA or DTPA-bisamide) or calcium chelate complexes (such as, for example, calcium DTPA, CaNaDTPA-bisamide), or, optionally, additions of calcium or sodium salts (for example, calcium chloride, calcium ascorbate, calcium gluconate or calcium lactate). Pharmaceutical compositions of the invention can be packaged for use in liquid form, or can be lyophilized.

[0081] For solid pharmaceutical compositions of the invention, conventional nontoxic solid pharmaceutically-acceptable carriers can be used; for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like.

[0082] For example, a solid pharmaceutical composition for oral administration can comprise any of the carriers and excipients listed above and 10-95%, preferably 25%-75%, of the at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising sequences encoding them). A pharmaceutical composition for aerosol (inhalational) administration can comprise 0.01-20% by weight, preferably 1%-10% by weight, of the at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising a sequence encoding the miR gene product or miR gene expression-inhibition compound) encapsulated in a liposome as described above, and a propellant. A carrier can also be included as desired; e.g., lecithin for intranasal delivery.

[0083] The pharmaceutical compositions of the invention can further comprise one or more anti-cancer agents. In a particular embodiment, the compositions comprise at least one miR gene product or miR gene expression-inhibition compound (or at least one nucleic acid comprising a sequence encoding the miR gene product or miR gene expression-inhibition compound) and at least one chemotherapeutic agent.

Chemotherapeutic agents that are suitable for the methods of the invention include, but are not limited to, DNA-alkylating agents, anti-tumor antibiotic agents, anti-metabolic agents, tubulin stabilizing agents, tubulin destabilizing agents, hormone antagonist agents, topoisomerase inhibitors, protein kinase inhibitors, HMG-CoA inhibitors, CDK inhibitors, cyclin inhibitors, caspase inhibitors, metalloproteinase inhibitors, antisense nucleic acids, triple-helix DNAs, nucleic acids aptamers, and molecularly-modified viral, bacterial and exotoxic agents. Examples of suitable agents for the compositions of the present invention include, but are not limited to, cytidine arabinoside, methotrexate, vincristine, etoposide (VP-16), doxorubicin (adriamycin), cisplatin (CDDP), dexamethasone, arglabin, cyclophosphamide, sarcolysin, methylnitrosourea, fluorouracil, 5-fluorouracil (5FU), vinblastine, camptothecin, actinomycin-D, mitomycin C, hydrogen peroxide, oxaliplatin, irinotecan, topotecan, leucovorin, carmustine, streptozocin, CPT-11, taxol, tamoxifen, dacarbazine, rituximab, daunorubicin, 1-β-D-arabinofuranosylcytosine, imatinib, fludarabine, docetaxel and FOLFOX4.

[0084] In one embodiment, the method comprises providing a test agent to a cell and measuring the level of at least one miR gene product associated with decreased expression levels in cancerous cells. An increase in the level of the miR gene product in the cell, relative to a suitable control (e.g., the level of the miR gene product in a control cell), is indicative of the test agent being an anti-cancer agent.

[0085] Suitable agents include, but are not limited to drugs (e.g., small molecules, peptides), and biological macromolecules (e.g., proteins, nucleic acids). The agent can be produced recombinantly, synthetically, or it may be isolated (i.e., purified) from a natural source. Various methods for providing such agents to a cell (e.g., transfection) are well known in the art, and several of such methods are described hereinabove. Methods for detecting the expression of at least one miR gene product (e.g., Northern blotting, *in situ* hybridization, RT-PCR, expression profiling) are also well known in the art. Several of these methods are also described herein.

[0086] **EXAMPLES**

[0087] The invention may be better understood by reference to the following non-limiting examples, which serve to illustrate but not to limit the present invention.

[0088] The data herein show that activated human mononuclear phagocytes and THP-1 cells release microvesicles that induce the survival and differentiation of freshly isolate monocytes. While not wishing to be bound by theory, the inventors herein believe that

under specific inflammatory diseases, the content of the microvesicles may be altered to rapidly induce a response. The data also show that microvesicles circulate in human peripheral blood. The circulating microvesicles regulate normal cellular homeostasis, and circulate instructions to distant cells during tissue injury and inflammation.

[0089] The microvesicles may serve as biomarkers for disease etiology and systemic mediators of the innate immune response. It is thus beneficial to be able to obtain similar information through the isolation of microvesicles in the peripheral blood instead of obtaining tissue through invasive procedures. Also, understanding the normal signature of microvesicles in the peripheral blood provides a basis for understanding events during acute inflammatory events.

[0090] As shown herein, aberrant macrophage differentiation contributes to disruption in immune homeostasis. Since monocyte maturation is induced by GM-CSF or M-CSF, the inventors initiated studies to understand the mechanisms and differences between GM-CSF- and M-CSF-mediated differentiation. The commitment to differentiate in response to GM-CSF but not M-CSF was rapid and irreversible (data not shown). Continuous GM-CSF stimulation was not required for this effect as only 4 hours of treatment induced macrophage differentiation. Similar observations were obtained in PMA-treated THP1 cells used as a model of macrophage differentiation.

[0091] Thus, the inventors determined that at least one factor was secreted upon inducing differentiation that either maintained signals or activated other cells to differentiate. Therefore, monocytes or THP1 cells were exposed to GM-CSF for 4h or PMA for 1h, respectively, after which cells were washed and placed in minimal media without stimulus. After 24 hours, the culture supernatants were collected and added to undifferentiated monocytes or THP1 cells. Notably, supernatants from PMA-treated THP1 cells or GM-CSF-treated monocytes differentiated monocytes and THP1 cells (data not shown).

[0092] Using the Bioplex suspension array system to detect up to 27 different cytokines in the culture supernatants, the inventors failed to detect a responsible cytokine. Since the inventors differentiated the growth factor-independent THP1 cell line with GM-CSF-stimulated monocyte supernatants, the inventors concluded that a cytokine/growth factor was not responsible for this effect. The inventors next investigated the possibility that microvesicles were secreted in the culture supernatant to mediate myeloid maturation.

[0093] As shown in **Figure 1**, monocytes treated with GM-CSF for 24 hours released

significant numbers of microvesicles (dark dots) in the culture supernatant compared to untreated monocytes (light dots).

[0094] Similarly, PMA-treated THP1 cells also secreted microvesicles during differentiation (data not shown). In particular, **Figure 1** shows the differentiation induced release of microvesicles from macrophages. Peripheral blood monocytes (PBM) were untreated (light) or treated with GM-CSF (dark) for 24 h. Cell-free supernatant was collected and ultracentrifuged. The vesicles were resuspended in PBS and analyzed for size on a flow cytometry. Prior to analysis, FSS and SSC parameters were adjusted using 2  $\mu$ m standard beads (not shown). Shown is representative data from three different donors.

[0095] Microvesicles from PMA-treated THP1 cells were purified and added to either freshly isolated monocytes or undifferentiated THP1 cells. The microvesicles alone induced macrophage differentiation in both cell types as indicated by morphology (see **Figures 2A-2C**) and expression of surface antigens (data not shown).

[0096] The content of these microvesicles has been analyzed. The inventors detected the presence of miRNAs in the microvesicles from PMA-treated THP1 cells (data not shown).

[0097] The inventors also evaluated circulating microvesicles and miRNA in the peripheral blood of normal volunteers. Based on size, the inventors found three subpopulations of microvesicles in the circulation (**Figure 3A**). Macrophage-derived microvesicles were detected using antibodies that detect mannose receptor (CD206) and MHC II (**Figure 3B**). Approximately 40% of the total microvesicles (gated region) in the plasma are derived from macrophages based on expression of either CD206 or MHCII (**Figure 3C**).

[0098] The inventors further determined whether miRNA are contained in the peripheral blood microvesicles. We detected expression of numerous miRNAs. The highest detected miRNAs are shown in **Figure 8** showing **Table III** (n=51).

[0099] Notably, miR-146 is undetectable in the peripheral blood whereas miR-155 expression was 80-fold lower than the highest expressing miRNA. Since both miR-146 and miR-155 were elevated in our IPF patient samples, but were low to undetectable in peripheral blood from normal donors, examination of circulating miRNAs may serve as a biomarker of disease.

[0100] It is now shown herein that circulating microvesicles contain miRNAs and that

circulating microvesicles can provide an avenue for the miRNAs to elicit cell-to-cell communication. The microvesicles housing miRNA can also provide insight into the genetic basis of disease and can serve as predictive biomarkers.

[00101] Also, microvesicles released during macrophage differentiation can mediate maturation of immature cells. Microvesicles collected during macrophage maturation mediate the differentiation and survival of human monocytes and contain RNA. Both miRNA and processed mRNA are responsible for the maturation signals imparted on immature cells.

[00102] **Example - Plasma**

[00103] Microvesicles are isolated from the plasma of normal healthy individuals. RNA is isolated from both the microvesicles and matched mononuclear cells and profiled for 420 known mature miRNAs by real-time PCR. Hierarchical cluster analysis of the data sets indicated significant differences in miRNA expression between peripheral blood mononuclear cells (PBMC) and plasma microvesicles.

[00104] We observed 104 and 75 miRNAs significantly expressed in the microvesicles and PBMC, respectively. Notably, 33 miRNAs were specifically expressed microvesicles compared to the PBMC. The miRNA were subjected to computational modeling to determine the biological pathways regulated by the detected miRNAs. The majority of the microRNAs expressed in the microvesicles from the blood were predicted to regulate cellular differentiation of blood cells and metabolic pathways. Interestingly, a select few microRNAs are predicted to be important modulators of immune function.

[00105] This example is the first to identify and define miRNA expression in circulating plasma microvesicles of normal subjects.

[00106] Recent evidence reveals that genetic exchange of mRNA and miRNA between cells can be accomplished through exosome-mediated transfer (PMID: 17486113). Microvesicles are small exosomes/vesicles of endocytic origin released by normal healthy or damaged cell types (PMID: 17337785, PMID: 17409393, PMID: 16791265). Microvesicles are shed from the plasma membrane into the extracellular environment to facilitate communication between cells. Despite their small size (50nm to 1μm) microvesicles are enriched in bioactive molecules and are suspected to contain nucleic acid and/or protein; these cell particles play a role in growth, differentiation and cancer progression (PMID: 16453000). In the peripheral blood, two-thirds of microvesicles are derived from platelets. Platelet-derived microvesicles play a role in

angiogenesis and the metastatic spread of cancers such as lung cancer (PMID: 15499615). Platelet-derived microvesicles induce an immune response upon regulating gene expression in hematopoietic, endothelial, and monocytic cells (PMID: 17378242, PMID: 17127485).

[00107] Interestingly, a connection between microvesicles and miRNA has been recently made. Recently, Valadi and colleagues reported that vesicles released from human and murine mast cell lines contain over 1200 mRNA and approximately 121 miRNA molecules (PMID: 17486113) In contrast, the present invention relates to naturally occurring human plasma and blood microvesicles containing microRNA that leads to biological effects *ex vivo*.

[00108] **Figure 8 – Table I** shows that microRNAs that are important in human diseases, including cancer and non-cancer applications. The microRNA molecules associated with increase expression in disease tissue but normally with low native or undetectable expression in human plasma microvesicles (**Table I**, shown in **Figure 6**) provides the opportunity to define changes in health and disease and may be effective biomarkers (Bold, Increase Expression Column). Similarly, normally abundant microRNAs may decrease in human plasma microvesicles to reflect the decrease observed in tissue (Bold, Decrease Expression Column).

[00109] Considerable evidence demonstrates the importance of miRNA as an inevitable cornerstone of the human genetic system. Employing the use of microvesicles to transfer genetic material would be an efficient transfer method within the human body. Microvesicular transport of miRNAs would enable communication at long distance.

#### [00110] METHODS

[00111] **Blood collection and microvesicle isolation.** Peripheral blood (40 cc) was collected in EDTA tubes from 24 females and 27 males healthy non-smoking Caucasian donors following informed consent. Collection of the blood occurred either between morning and early afternoon. The median age for female donors was 29 as well as for male donors. The peripheral blood was diluted 1:1 with sterile low endotoxin PBS, layered over ficoll-hypaque (d=1.077), and centrifuged as previously described (PMID: 16931806). The mononuclear cell fraction was washed once in PBS. The microvesicles were purified from the plasma. Briefly, the vesicles were concentrated by centrifugation at 160,000x g for 1hr at 4°C (PMID: 10648405).

[00112] **RNA Extraction.** Total RNA was isolated by Trizol (Invitrogen, Carlsbad,

CA) extraction method. To increase the yield of small RNAs, the RNA was precipitated overnight. RNA concentration was determined and RNA integrity was determined by capillary electrophoresis on an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc, Santa Clara, CA). For RNA isolated from mononuclear cells, only a RNA integrity number (RIN)  $\geq 9$  was used. Since the intact 18s and 28s rRNA was variable in the microvesicles, the RIN was not a constraint for these samples.

[00113] **miRNA profiling by quantitative PCR.** The expression of 420 mature human miRNAs was profiled by real-time PCR. RNA (50 ng) was converted to cDNA by priming with a mixture of looped primers to 420 known human mature miRNAs (MegaPlex kit, Applied Biosystems, Foster City, CA) using previously published reverse transcription conditions (PMID: 18158130). As there is no known control miRNA in microvesicles, several internal controls were examined. Primers to the internal controls, small nucleolar (sno)RNA U38B, snoRNA U43, small nuclear (sn)RNA U6 as well as 18S and 5S rRNA were included in the mix of primers.

[00114] The expression was profiled using an Applied Biosystems 7900HT real-time PCR instrument equipped with a 384 well reaction plate. Liquid-handling robots and the Zymak Twister robot were used to increase throughput and reduce error. Real-time PCR was performed using standard conditions.

[00115] **Flow Cytometry.** Peripheral blood microvesicles were directly immunostained from plasma without concentration by centrifugation. To determine the cellular origin, 0.5 cc plasma was immunostained per panel of antibodies. Panel I contained antibodies recognizing CD66b-FITC (neutrophil), CD202b (Tie2)-PE (endothelial), CD206 PE-Cy5 (macrophage/dendritic), CD79a-APC (B-cell), and CD14 Pe-Cy7 (monocyte). Panel II contained antibodies to CD41a-PE-Cy5 (platelet), CCR2-APC (monocyte), CCR3-PE (dendritic cell), CCR5-PE-Cy7 (macrophage), and CD3-Alexa 610 (T-cell). Panel III contained isotype control antibodies. The samples were analyzed on BD Aria flow cytometer (BD Biosciences San Jose, CA). Data was expressed as percent of gated cells.

[00116] **Statistical analysis.** To reduce background noise, the miRNAs in which 80% of individual observations had a raw CT score greater than 35 were not considered during the data analysis. The internal controls (18S, 5S, snoRNA U38B, snoRNA U43, and snRNA U6) were highly variable in the plasma microvesicles as well as significantly different levels of expression in plasma microvesicles versus peripheral blood

mononuclear cells (PBMC).

[00117] Thus, to reduce bias caused by using a certain miRNA as a normalization correction factor and to reduce the sample variations among RT-PCR arrays, the miRNAs were compared between plasma microvesicles and PBMC based on their relative expression to the overall miRNA expression on each array using median normalization analysis (PMID: 16854228). Controlling gender and age of the donors, linear mixed models were used to estimate the difference of specific miRNA between plasma microvesicles and PBMC. Fold-change was calculated based on the estimated mean difference.

[00118] Heat maps were generated using the miRNA that passed the filtering criteria for each tissue and miRNAs were subjected to hierarchical clustering based on their relative mean expression. miRNA expression was also ranked based on their raw CT score for plasma microvesicles and PBMC. Additional statistical analysis such as ANOVA was performed to determine miRNAs that are significant expressed between the two treatment groups

[00119] **Pathway analysis and prediction.** Predicted miRNAs targets were determined using miRanda ([microrna.sanger.ac.uk/targets/v5/](http://microrna.sanger.ac.uk/targets/v5/)). Based on the miRanda algorithm, a score is generated for each target, only scores greater than 17 were furthered analyzed using Ingenuity Pathway Analysis software (Ingenuity Systems, Redwood City, CA). Using this software, canonical pathways were determined based on targets of the miRNAs. The dataset was examined to determine associated pathways based on gene ontology of miRNA's targets.

[00120] **RESULTS**

[00121] **Peripheral blood microvesicle subpopulations**

[00122] Initially, we examined the cellular origin of microvesicles within the peripheral blood of normal healthy individuals. Using flow cytometry, we found that the majority of the peripheral blood microvesicles are platelet-derived (**Figure 4**), as previously reported (PMID: 10648405).

[00123] We also observed a second large population of microvesicles that were derived from mononuclear cell phagocyte lineage. This population was immunostained with antibodies that detected surface antigens on mononuclear phagocytes. Notably, only a small percentage of the peripheral blood microvesicles were derived from T-cells and neutrophils. We failed to detect vesicles that originated from B-cells (data not shown). Of

interest, we detected a small subpopulation of microvesicles that expressed surface antigens from endothelial cells.

[00124] **miRNA expression in plasma microvesicles and PBMC**

[00125] To test whether miRNAs are contained in the microvesicle compartment within the peripheral blood to enable communication and influence genetic changes between different tissues within the body, we performed miRNA profiling on the purified microvesicles from the plasma. We analyzed all subpopulations of microvesicles from 51 non-smoking healthy individuals comprising of 27 males and 24 females. In order to determine whether there would be differences in miRNA expression between microvesicles and PBMC, we also purified the PBMC from each donor. Real-time PCR analysis was performed to examine the expression of 420 miRNAs. The filtered data was subjected to hierachal cluster analysis comparing the miRNA expression profile between the PBMC and plasma samples (**Figure 5A**).

[00126] All but three PBMC samples clustered separately from the microvesicle samples, indicating that the miRNA expression profile between the two groups was significantly different. Based on filtering criteria to reduce background noise, we found 104 and 75 miRNAs expressed in the microvesicles and PBMC samples, respectively (**Figures 5B and 5C**).

[00127] Of these miRNAs, 71 were shared among each sample group (**Figure 5D**). Notably, only two miRNAs miR-031 and 29c were expressed solely in the PBMC samples whereas four miRNAs (miR -127, -134, -485-5p, and -432 ) were uniquely expressed in the plasma fraction. All 104 miRNAs that are normally expressed in the plasma are shown (**Table II**, shown in **Figure 7**).

[00128] **Age and gender effects**

[00129] We did not observe age and/or gender effects in miRNA expression from either sample group. Notably, the median age for both female and male donors was 29 years. The oldest individual was 58 years old, while the youngest was 21 years of age. Thus, we furthered stratified the data to examine differences. Examination between age-matched samples did not reveal any significant effects on miRNA expression between PBMC and microvesicles samples. While controlling gender, we also compared the upper quartile of age with the lower quartile of age, mean age for each group was  $48.9 \pm 6.2$  and  $21.9 \pm 1.2$ , respectively. However, we failed to detect significant differences in miRNA expression between the samples sets based on age (data not shown).

[00130] **Comparison of miRNA expression in PBMC and microvesicles**

[00131] Shown in **Table III, Figure 8**, is the top ten expressed miRNAs in the plasma microvesicles and the PBMC from all individuals. For plasma the top ten expressed miRNAs in descending order are miR-223, -484, -191, -146a, -016, -026a, -222, -024, -126, and -32. Whereas, miR-223, -150, -146b, -016, -484, -146a, -191, -026a, -019b, and -020a were highly expressed in the PBMC. The top ten expressed miRNAs in the microvesicles were detected in 100% of the individuals. However, in the PBMC samples, all but miR-150 (98% of donors) and miR-484 (89% of donors) were observed in 100% of the individuals.

[00132] We also found that six of these miRs (miR-223, miR-484, miR-191, miR-146a, miR-26a, and miR-16) are shared among the top ten in both PBMC and microvesicles. Notably, miR-223 is the most prominently expressed miR in both compartments. Based on ranking analysis for each individual donor to determine the frequency in which the specific miRNA appeared in the top ten expressed miRNA, miR-223 had a frequency of 100% in both PBMC and microvesicles. Despite expression of miR-486 being the in the top ten expressed miRNAs in the plasma microvesicles, this miRNA was found to be expressed in the top ten of only 20% of the individuals profiled. Interestingly, the highly expressed miRNAs in the plasma microvesicles were not identified as tissue-specific miRs.

[00133] We further examined the collective function of the miRs in microvesicles and PBMC with a ranking score greater than arbitrary values of >66% and >88%, respectively (natural cut-offs from the data set). Based on this criterion, we further examined the top 9 ranked miRs from the microvesicles and PBMC samples. Thus, we analyzed the combined function of miR-223, -484, -191, -146a, -016, -026a, -222, -024, and -126 found in the plasma. For PBMC, we examined the combined function of the following miRNAs, miR-223, -150, -146b, -016, -484, -146a, -191, -026a, and -019b. Using the Sanger miRBase Target version 5, we found 1578 predicted targets of the combined miRs for the plasma microvesicles (data not shown). These combined targets were subjected to computational analysis to determine the pathways that they collectively regulate. Using the Ingenuity Pathway Analysis (IPA) software, we found canonical pathways involved in metabolism and regulation of the acquired immune system were highly regulated by the expression of these shown in miRNAs (**Table IV**, shown in **Figure 9**, top

[00134] Of the nine miRNAs examined from the PBMC fraction, we found 1857 predicted mRNA targets (data not shown). Ultimately the top five canonical pathways regulated by these miRNAs are various amino acid and lipid metabolic pathways, among others (**Table IV**, shown in **Figure 9**, top). We also found common predicted targets from Sanger miRBase and TargetScan and determined their function (**Table IV**, shown in **Figure 9**, bottom).

[00135] We next examined which miRNAs were differentially expressed between microvesicles and PBMC. We found 20 miRNAs had more than a three-fold increase in expression in the PBMC fraction compared to the microvesicles samples (**Table V**, shown in **Figure 10**). In contrast, 15 miRNAs were significantly expressed in the plasma microvesicles compared to PBMC.

[00136] **Figure 11: Table VI** shows the average normalized data for all miRNAs (detector name) expressed in the PBMC and the plasma with standard deviation for each.

## [00137] DISCUSSION

[00138] In these examples, the inventors now show that miRs circulate in microvesicles under normal homeostatic conditions in the peripheral blood. Here, we demonstrate 104 miRs expressed in plasma microvesicles and miR expression was significantly different from PBMC. To date, numerous studies demonstrate the ability of miRs to regulate many cellular functions. However, these studies largely imply that the miR stays within its host cell to elicit an effect (PMID: 17923084). Our data indicates that the miRNAs contained in the microvesicles may be communication signals to distant cells to regulate cellular homeostasis.

[00139] These miRNAs in the microvesicles may circulate to different tissue targets. Further examination of the highest expressed miRNAs in the plasma microvesicles, demonstrate that many of these function to regulate hematopoiesis and cellular differentiation programs (**Table III**, shown in **Figure 8**). For instances, expression of miR-223 regulates myeloid, granulocytic and osteoclasts differentiation (PMID: 18278031, PMID: 17471500, PMID: 16325577). It also appears to have a role in hematopoietic stem cell proliferation (PMID: 18278031). Interestingly, miR-223 is loss in acute myelogenous leukemia (AML) (PMID: 18056805). In contrast, downregulation of miR-126 occurs during megakaryocyte differentiation (PMID: 16549775). Notably, expression of miR-24 is regulated by TGF- $\beta$  which is a potent positive and negative regulator of hematopoiesis (PMID: 16123808, PMID: 18353861). Both miR-24 and miR-

16 expressed in the microvesicles regulates red cell production (PMID: 17906079, PMID: 17976518), while miR-16 also modulates lymphoid development (PMID: 16616063). Loss of miR-16 expression has been extensively examined in chronic lymphocytic leukemia (CLL) (PMID: 17327404, PMID: 17351108 ).

[00140] Many miRs expressed in the plasma microvesicles also regulate the progression of the cell cycle proteins (PMID: 18365017 PMID: 17914108). MiR-222 targets p27Kip1 (PMID: 17914108) while miR-24 suppresses p16 (INK4a) (PMID: 18365017). Increased expression of miR-16 results in the accumulation of cells in G0/G1 phase of the cell cycle (PMID: 16123808). In contrast, expression of miR-126 in breast cancer cells increases cellular proliferation and tumor growth but inhibits metastases (PMID: 18185580). This occurs through the regulation of vascular cell adhesion molecule-1 (VCAM1) (PMID: 18227515).

[00141] Unlike the other miRs highly expressed in the plasma microvesicles, miR-146a appears to function at a different level. While it has been suggested that miR-146a acts as a tumor suppressor and loss of this miR is associated with the development of prostate cancer (PMID: 18174313), miR-146a also modulates immune function (PMID: 16885212, PMID: 18057241). It is possible that expression of this miR in the plasma microvesicles defines immune regulatory function (**Table IV**, shown in **Figure 9**).

[00142] Based on IPA analysis examining gene ontology of targets, the top associated networks predicted to be influenced by miR-146a expression is cellular proliferation, immune and lymphatic system development and function. In addition, this miR is predicted to regulate innate immune responses. From the analysis, we found that LPS/IL-1 and toll-like receptor signaling are among the top five canonical pathways predicted to be regulated by this miR-146a.

[00143] To date, there is no known function for miR-484 or miR-486. Similar to miR-146a, miR-484 and miR-486 appear to function as a modulator of immune responsiveness. Notably, miR-484 is the second highest expressed miR in the microvesicles fraction based on relative expression. Prediction modeling indicates that this miR has multiple functions. Like many of the other miRs expressed in the microvesicles, miR-484 is predicted to regulate hematopoiesis. In particular, NK cell signaling and IL-4 signaling pathways are predicted to be targets of miR-484, while miR-486 is proposed to regulate antigen presentation. In addition, miR-486 appears to regulate cell differentiation, proliferation and growth.

[00144] While we detected 104 miRs in the plasma microvesicles, there were many that were undetectable from the total miRs profiled. Undetectable miRs in plasma microvesicles may also serve as disease biomarkers. Recently, Lawrie et al. reported that miRs were detected in the plasma of patients with B-cell lymphoma (PMID: 18318758). This study, indicated that miR-155, miR-210 and miR-21 were elevated in the plasma from these patients and miR-21 correlated with relapse. Based on this study, we detected miR-155 and miR-21 in normal individuals, but did not find miR-210. Interestingly, we found that 75% of individuals expressed miR-155 and 60% expressed miR-21 in the plasma (data not shown).

[00145] Thus, for these miRs to be used as predictive markers of disease, each individual would require a baseline prior to detection of disease. Thus, expression of miR-210 may serve as a better marker of B-cell lymphoma. Additional relationships may exist. For instance, miR-203 was undetectable in plasma microvesicles. Elevated expression of this miR is associated with bladder carcinoma and colon adenocarcinoma and may be thus used as a biomarker (PMID: 18230780, PMID: 17826655 ).

[00146] A converse relation may exist for plasma miRs that are normally expressed then lost with disease. For example, in acute lymphocytic leukemia (ALL), miR-223 is downregulated (PMID: 18056805). Since miR-223 is the most prominent miR expressed in the plasma microvesicles, its reduced expression may be useful as a diagnostic marker in ALL. In addition, miRs-15a/16 are lost or downregulated in chronic lymphocytic leukemia (CLL) (PMID: 18362358). While we found miR-16 was expressed in all healthy individuals that were examined, miR-15a was only expressed in 44% of the individuals profiled (data not shown).

[00147] It is of interest that we did not detect tissue specific miRNAs in the blood of normal individuals (PMID: 18025253). The majority of the microvesicles from normal individuals are derived from blood cells. We did detect a small percentage of microvesicles derived from endothelial cells. The endothelial-derived microvesicles may increase upon endothelial cell damage. Likewise, the detection of tissue specific miRs and microvesicles in the peripheral blood may be a frequent event upon tissue damage. Since tumors produce microvesicles (PMID: 16283305), these may be detected in the peripheral blood.

[00148] While it has been reported that miRs are detected in the plasma (PMID: 18318758), this is the first study to characterize all known miRs from the plasma. In this

study, we controlled race as a factor.

[00149] Testing the presence, absence or alterations in levels of miRs in peripheral fluids and/or blood can be useful as biomarkers to examine various diseases, to identify unique miRNA profiles, and to be a predictor of disease. The circulating miRs contained in the microvesicles have a vital function in regulating homeostasis production of blood cells as well as metabolic functions

[00150] The relevant teachings of all publications cited herein that have not explicitly been incorporated by reference, are incorporated herein by reference in their entirety. While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

[00151] While the invention has been described with reference to various and preferred embodiments, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted for elements thereof without departing from the essential scope of the invention. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from the essential scope thereof. Therefore, it is intended that the invention not be limited to the particular embodiment disclosed herein contemplated for carrying out this invention, but that the invention will include all embodiments falling within the scope of the claims.

[00152] The miRs of interest are listed in public databases. In certain preferred embodiments, the public database can be a central repository provided by the Sanger Institute [www.http://microrna.sanger.ac.uk/sequences/](http://microrna.sanger.ac.uk/sequences/) to which miR sequences are submitted for naming and nomenclature assignment, as well as placement of the sequences in a database for archiving and for online retrieval via the world wide web. Generally, the data collected on the sequences of miRs by the Sanger Institute include species, source, corresponding genomic sequences and genomic location (chromosomal coordinates), as well as full length transcription products and sequences for the mature fully processed miRNA (miRNA with a 5' terminal phosphate group). Another database can be the GenBank database accessed through the National Center for Biotechnology Information (NCBI) website, maintained by the National Institutes of Health and the National Library of Medicine. These databases are fully incorporated herein by reference.

miR * Biogenesis byproducts that are at low level, function unknown	miRBase Mature Sequence Accession #	Mature Sequence	SEQ ID NO
hsa-let-7a*	MIMAT0004481	CUAUACAAUCUACUGUCUUUC	<b>1</b>
hsa-let-7a-1	MIMAT0000062	UGAGGUAGUAGGUUGUAUAGUU	<b>2</b>
hsa-let-7a-2	MIMAT0000062	UGAGGUAGUAGGUUGUAUAGUU	<b>3</b>
hsa-let-7a-3	MIMAT0000062	UGAGGUAGUAGGUUGUAUAGUU	<b>4</b>
hsa-let-7b	MIMAT0000063	UGAGGUAGUAGGUUGUGUGGUU	<b>5</b>
hsa-let-7b*	MIMAT0004482	CUAUACAAACCUACUGCCUUCCC	<b>6</b>
hsa-let-7c	MIMAT0000064	UGAGGUAGUAGGUUGUAUGGUU	<b>7</b>
hsa-let-7c*	MIMAT0004483	UAGAGUUACACCCUGGGAGUUA	<b>8</b>
hsa-let-7d	MIMAT0000065	AGAGGUAGUAGGUUGCAUAGUU	<b>9</b>
hsa-let-7d*	MIMAT0004484	CUAUACGACCUGCUGCCUUUCU	<b>10</b>
hsa-let-7e	MIMAT0000066	UGAGGUAGGAGGUUGUAUAGUU	<b>11</b>
hsa-let-7e*	MIMAT0004485	CUAUACGGCCUCCUAGCUUUCC	<b>12</b>
hsa-let-7f-1	MIMAT0000067	UGAGGUAGUAGAUUGUAUAGUU	<b>13</b>
hsa-let-7f-1*	MIMAT0004486	CUAUACAAUCUAUUGCCUUCCC	<b>14</b>
hsa-let-7f-2	MIMAT0000067	UGAGGUAGUAGAUUGUAUAGUU	<b>15</b>
hsa-let-7f-2*	MIMAT0004487	CUAUACAGCUACUGUCUUUCC	<b>16</b>
hsa-let-7g	MIMAT0000414	UGAGGUAGUAGUUUGUACAGUU	<b>17</b>
hsa-let-7g*	MIMAT0004584	CUGUACAGGCCACUGCCUUGC	<b>18</b>
hsa-let-7i	MIMAT0000415	UGAGGUAGUAGUUUGUGCUGUU	<b>19</b>
hsa-let-7i*	MIMAT0004585	CUGCGCAAGCUACUGCCUUGC	<b>20</b>
hsa-mir-009-1	MIMAT0000441	UCUUUGGUUAUCUAGCUGUAUGA	<b>21</b>
hsa-mir-009-1*	MIMAT0000442	AUAAAGCUAGAUACCAGAAAGU	<b>22</b>
hsa-mir-009-2	MIMAT0000441	UCUUUGGUUAUCUAGCUGUAUGA	<b>23</b>
hsa-mir-009-3	MIMAT0000441	UCUUUGGUUAUCUAGCUGUAUGA	<b>24</b>
hsa-mir-010a	MIMAT0000253	UACCCUGUAGAUCCGAAUUUGUG	<b>25</b>
hsa-mir-010a*	MIMAT0004555	CAAAUUCGUACUAGGGAAUA	<b>26</b>
hsa-mir-015a	MIMAT0000068	UAGCAGCACAUAAUGGUUGUG	<b>27</b>
hsa-mir-015b	MIMAT0000417	UAGCAGCACAUCAUGGUUACA	<b>28</b>
hsa-mir-015b*	MIMAT0004586	CGAAUCAUUAUUUGCUGCUCUA	<b>29</b>
hsa-mir-016-1	MIMAT0000069	UAGCAGCACGUAAAUAUUGGCG	<b>30</b>
hsa-mir-016-1*	MIMAT0004489	CCAGUAUUAACUGUGCUGCUGA	<b>31</b>
hsa-mir-016-2	MIMAT0000069	UAGCAGCACGUAAAUAUUGGCG	<b>32</b>
hsa-mir-016-2*	MIMAT0004518	CCAAUAAUACUGUGCUGCUCUUA	<b>33</b>
hsa-mir-017-3-p	MIMAT0000071	ACUGCAGUGAAGGCACUUGUAG	<b>34</b>
hsa-mir-017-5-p	MIMAT0000070	CAAAGUGCUUACAGUGCAGGUAG	<b>35</b>
hsa-mir-018a	MIMAT0000072	UAAGGUGCAUCUAGUGCAGAUAG	<b>36</b>
hsa-mir-018a*	MIMAT0002891	ACUGCCCUAAGUGCUCUUCUGG	<b>37</b>
hsa-mir-019a	MIMAT0000073	UGUGCAAAUCUAUGCAAAACUGA	<b>38</b>
hsa-mir-019b-1	MIMAT0000074	UGUGCAAAUCCAUGCAAAACUGA	<b>39</b>
hsa-mir-019b-1*	MIMAT0004491	AGUUUUGCAGGUUUGCAUCCAGC	<b>40</b>
hsa-mir-019b-2	MIMAT0000074	UGUGCAAAUCCAUGCAAAACUGA	<b>41</b>

hsa-mir-019b-2*	MIMAT0004492	AGUUUUGCAGGUUUGCAUUCA	42
hsa-mir-020a	MIMAT0000075	UAAAGUGCUUAUAGUGGCAGGUAG	43
hsa-mir-020a*	MIMAT0004493	ACUGCAUUAUGAGCACUUAAG	44
hsa-mir-020b	MIMAT0001413	CAAAGUGCUCAUAGUGGCAGGUAG	45
hsa-mir-021	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA	46
hsa-mir-021*	MIMAT0004494	CAACACCAGUCGAUGGGCUGU	47
hsa-mir-023a	MIMAT0000078	AUCACAUUGCCAGGGAUUCC	48
hsa-mir-023a*	MIMAT0004496	GGGGUUCCUGGGGAUGGGAUUU	49
hsa-mir-023b	MIMAT0004587	UGGGUUCCUGGCAUGCUGAUUU	50
hsa-mir-024-1	MIMAT0000080	UGGCUCAGUUCAGCAGGAACAG	51
hsa-mir-024-1*	MIMAT0000079	UGCCUACUGAGCUGAUACAGU	52
hsa-mir-024-2	MIMAT0000080	UGGCUCAGUUCAGCAGGAACAG	53
hsa-mir-024-2*	MIMAT0004497	UGCCUACUGAGCUGAAACACAG	54
hsa-mir-025	MIMAT0000081	CAUUGCACUUGUCUCGGUCUGA	55
hsa-mir-025*	MIMAT0004498	AGGCGGAGACUUGGGCAAUUG	56
hsa-mir-026a-1	MIMAT0000082	UUCAAGUAUCCAGGAUAGGCU	57
hsa-mir-026a-1*	MIMAT0004499	CCUAUUCUUGGUACUUGCACG	58
hsa-mir-026a-2	MIMAT0000082	UUCAAGUAUCCAGGAUAGGCU	59
hsa-mir-026a-2*	MIMAT0004681	CCUAUUCUUGAUUACUUGUUUC	60
hsa-mir-026b	MIMAT0000083	UUCAAGUAUUUCAGGAUAGGU	61
hsa-mir-026b*	MIMAT0004500	CCUGUUCUCCAUUACUUGGCUC	62
hsa-mir-027a	MIMAT0000084	UUCACAGUGGCUAAGUUCCGC	63
hsa-mir-027a*	MIMAT0004501	AGGGCUUAGCUGCUUGUGAGCA	64
hsa-mir-027b	MIMAT0000419	UUCACAGUGGCUAAGUUCUGC	65
hsa-mir-027b*	MIMAT0004588	AGAGCUUAGCUGAUUGGUGAAC	66
hsa-mir-028-3p	MIMAT0004502	CACUAGAUUGUGAGCUCCUGGA	67
hsa-mir-028-5p	MIMAT0000085	AAGGAGCUCACAGCUAUUGAG	68
hsa-mir-029a	MIMAT0000086	UAGCACCAUCUGAAAUCGGUUA	69
hsa-mir-029a*	MIMAT0004503	ACUGAUUUCUUUUGGUUCAG	70
hsa-mir-029b-1	MIMAT0000100	UAGCACCAUUUGAAAUCAGUGUU	71
hsa-mir-029b-1*	MIMAT0004514	GCUGGUUUCAUUAUGGUGGUUUAGA	72
hsa-mir-029b-2	MIMAT0000100	UAGCACCAUUUGAAAUCAGUGUU	73
hsa-mir-029b-2*	MIMAT0004515	CUGGUUUCACAUGGUGGCCUUAG	74
hsa-mir-029b-3	MIMAT0000100	UAGCACCAUUUGAAAUCAGUGUU	75
hsa-mir-029c	MIMAT0000681	UAGCACCAUUUGAAAUCGGUUA	76
hsa-mir-030a	MIMAT0000087	UGUAAACAUCCUCGACUGGAAG	77
hsa-mir-030a*	MIMAT0000088	CUUUCAGUCGGAUGUUUGCAGC	78
hsa-mir-030b	MIMAT0000420	UGUAAACAUCUCACACUCAGCU	79
hsa-mir-030b*	MIMAT0004589	CUGGGAGGUGGAUGUUUACUUC	80
hsa-mir-030c-1	MIMAT0000244	UGUAAACAUCUCACACUCUCAGC	81
hsa-mir-030c-2	MIMAT0000244	UGUAAACAUCUCACACUCUCAGC	82
hsa-mir-030c-2*	MIMAT0004550	CUGGGAGAAGGCUGUUUACUCU	83
hsa-mir-030d	MIMAT0000245	UGUAAACAUCUCGACUGGAAG	84
hsa-mir-030d*	MIMAT0004551	CUUUCAGUCAGAUGUUUGCUGC	85
hsa-mir-031	MIMAT0000089	AGGCAAGAUGCUGGCAUAGCU	86
hsa-mir-031*	MIMAT0004504	UGCUAUGCCAACAUAUUGCCAU	87

hsa-mir-032	MIMAT0000090	UAUUGCACAUUACUAAGUUGCA	88
hsa-mir-032*	MIMAT0004505	CAAUUUAGUGUGUGUGAUUU	89
hsa-mir-034a	MIMAT0000255	UGGCAGUGUCUUAGCUGGUUGU	90
hsa-mir-034a*	MIMAT0004557	CAAUCAGCAAGUAUACUGCCU	91
hsa-mir-092a-1	MIMAT0000092	UAUUGCACUUGUCCGGCCUGU	92
hsa-mir-092a-1*	MIMAT0004507	AGGUUGGGAUCGGUUGCAAUGCU	93
hsa-mir-093	MIMAT0000093	CAAAGUGCUGUUCGUGCAGGUAG	94
hsa-mir-093*	MIMAT0004509	ACUGCUGAGCUAGCACUUCCG	95
hsa-mir-095	MIMAT0000094	UUCAACGGGUUUUAUUGAGCA	96
hsa-mir-096	MIMAT0000095	UUUGGCACUAGCACAUUUUGCU	97
hsa-mir-096*	MIMAT0004510	AAUCAUGUGCAGUGCCAAUAUG	98
hsa-mir-098	MIMAT0000096	UGAGGUAGUAAGUUGUAUUGUU	99
hsa-mir-099b	MIMAT0000689	CACCGUAGAACCGACCUUGCG	100
hsa-mir-099b*	MIMAT0004678	CAAGCUCGUGUCUGUGGGUCCG	101
hsa-mir-100	MIMAT0000098	AACCGUAGAUCCGAACUUGUG	102
hsa-mir-100*	MIMAT0004512	CAAGCUUGUAUCUAUAGGUAUG	103
hsa-mir-103-1	MIMAT0000101	AGCAGCAUUGUACAGGGCUAUGA	104
hsa-mir-103-2	MIMAT0000101	AGCAGCAUUGUACAGGGCUAUGA	105
hsa-mir-105-1	MIMAT0000102	UCAAAUGCUCAGACUCCUGUGGU	106
hsa-mir-105-1*	MIMAT0004516	ACGGAUGUUUGAGCAUGUGCUA	107
hsa-mir-105-2	MIMAT0000102	UCAAAUGCUCAGACUCCUGUGGU	108
hsa-mir-105-2*	MIMAT0004516	ACGGAUGUUUGAGCAUGUGCUA	109
hsa-mir-106a	MIMAT0000103	AAAAGUGCUCUACAGUGCAGGUAG	110
hsa-mir-106a*	MIMAT0004517	CUGCAAUGUAAGCACUUUCUAC	111
hsa-mir-106b	MIMAT0000680	UAAAGUGCUGACAGUGCAGAU	112
hsa-mir-106b*	MIMAT0004672	CCGCACUGUGGGUACUUGCUGC	113
hsa-mir-107	MIMAT0000104	AGCAGCAUUGUACAGGGCUAUCA	114
hsa-mir-122	MIMAT0000421	UGGAGUGUGACAAUGGUGUUUG	115
hsa-mir-122*	MIMAT0004590	AACGCCAUUAUCACACUAAAUA	116
hsa-mir-125a-3p	MIMAT0004602	ACAGGUGAGGUUCUUGGGAGCC	117
hsa-mir-125a-5p	MIMAT0000443	UCCCUGAGACCCUUUAACCUGUGA	118
hsa-mir-125b-1	MIMAT0000423	UCCCUGAGACCCUAACUUGUGA	119
hsa-mir-125b-1*	MIMAT0004592	ACGGGUUAGGCUCUUGGGAGCU	120
hsa-mir-125b-2	MIMAT0000423	UCCCUGAGACCCUAACUUGUGA	121
hsa-mir-125b-2*	MIMAT0004603	UCACAAGUCAGGCUCUUGGGAC	122
hsa-mir-126	MIMAT0000445	UCGUACCGUGAGUAUAUAGCG	123
hsa-mir-126*	MIMAT0000444	CAUUAUUACUUUUGGUACGCG	124
hsa-mir-127-3p	MIMAT0000446	UCGGAUCCGUCUGAGCUUGGCU	125
hsa-mir-127-5p	MIMAT0004604	CUGAAGCUCAGAGGGCUCUGAU	126
hsa-mir-128-1	MIMAT0000424	UCACAGUGAACCGGUCUCUUU	127
hsa-mir-128-2	MIMAT0000424	UCACAGUGAACCGGUCUCUUU	128
hsa-mir-130a	MIMAT0000425	CAGUGCAAUGUAAAAGGGCAU	129
hsa-mir-130a*	MIMAT0004593	UUCACAUUGUGCUACUGUCUGC	130
hsa-mir-130b	MIMAT0000691	CAGUGCAAUGAUGAAAGGGCAU	131
hsa-mir-130b*	MIMAT0004680	ACUCUUUCCCCUGUUGCACUAC	132
hsa-mir-132	MIMAT0000426	UAACAGUCUACAGCCAUGGUUG	133

hsa-mir-132*	MIMAT0004594	ACCGUGGCUUUCGAUUGUUACU	134
hsa-mir-133a-1	MIMAT0000427	UUUGGUCCCCUUCAACCAGCUG	135
hsa-mir-133a-2	MIMAT0000427	UUUGGUCCCCUUCAACCAGCUG	136
hsa-mir-133b	MIMAT0000770	UUUGGUCCCCUUCAACCAGCUA	137
hsa-mir-134	MIMAT0000447	UGUGACUGGUUGACCAGAGGGG	138
hsa-mir-135b	MIMAT0000758	UAUGGCUUUUCAUCCUAUGUGA	139
hsa-mir-135b*	MIMAT0004698	AUGUAGGGCUAAAAGCCAUGGG	140
hsa-mir-140-3p	MIMAT0004597	UACCACAGGUAGAACACCGG	141
hsa-mir-140-5p	MIMAT0000431	CAGUGGUUUUACCCUAUGGUAG	142
hsa-mir-142-3p	MIMAT0000434	UGUAGUGUUUCCUACUUUAUGGA	143
hsa-mir-142-5p	MIMAT0000433	CAUAAAGUAGAAAGCACUACU	144
hsa-mir-143	MIMAT0000435	UGAGAUGAAGCACUGUAGCUC	145
hsa-mir-143*	MIMAT0004599	GGUGCAGUGCUGCAUCUCUGGU	146
hsa-mir-145	MIMAT0000437	GUCCAGUUUUCCCAGGAUICCU	147
hsa-mir-145*	MIMAT0004601	GGAUUCCUGGAAAUACUGUUCU	148
hsa-mir-146a	MIMAT0000449	UGAGAACUGAAUUCCAUAGGGU	149
hsa-mir-146a*	MIMAT0004608	CCUCUGAAAUCAGUUCUUCAG	150
hsa-mir-146b-3p	MIMAT0004766	UGCCCUGUGGACUCAGUUCUGG	151
hsa-mir-146b-5p	MIMAT0002809	UGAGAACUGAAUUCCAUAGGCU	152
hsa-mir-147	MIMAT0000251	GUGUGUGGAAAUGCUUCUGC	153
hsa-mir-148a	MIMAT0000243	UCAGUGCACUACAGAACUUUGU	154
hsa-mir-148a*	MIMAT0004549	AAAGUUCUGAGACACUCCGACU	155
hsa-mir-148b	MIMAT0000759	UCAGUGCAUCACAGAACUUUGU	156
hsa-mir-148b*	MIMAT0004699	AAGUUCUGUUUAACACUCAGGC	157
hsa-mir-149	MIMAT0000450	UCUGGCUCCGUGUCUUCACUCC	158
hsa-mir-149*	MIMAT0004609	AGGGAGGGACGGGGCUGUGC	159
hsa-mir-150	MIMAT0000451	UCUCCCAACCCUUGUACCAGUG	160
hsa-mir-150*	MIMAT0004610	CUGGUACAGGCCUGGGGGACAG	161
hsa-mir-151-3p	MIMAT0000757	CUAGACUGAAGCUCCUUGAGG	162
hsa-mir-151-5p	MIMAT0004697	UCGAGGAGCUCACAGUCUAGU	163
hsa-mir-155	MIMAT0000646	UUAAUGCUAAUCGUGAUAGGGGU	164
hsa-mir-155*	MIMAT0004658	CUCCUACAUUUAGCAUUAACA	165
hsa-mir-181a-1	MIMAT0000256	AACAUUCAACGCUGUCGGUGAGU	166
hsa-mir-181a-1*	MIMAT0000270	ACCAUCGACCGUUGAUUGUACC	167
hsa-mir-181a-2	MIMAT0000256	AACAUUCAACGCUGUCGGUGAGU	168
hsa-mir-181a-2*	MIMAT0004558	ACCACUGACCGUUGACUGUACC	169
hsa-mir-181b-1	MIMAT0000257	AACAUUCAUUGCUGUCGGUGGGU	170
hsa-mir-181b-2	MIMAT0000257	AACAUUCAUUGCUGUCGGUGGGU	171
hsa-mir-181d	MIMAT0002821	AACAUUCAUUGUUGUCGGUGGGU	172
hsa-mir-182	MIMAT0000259	UUUGGCAAUGGUAGAACUCACACU	173
hsa-mir-182*	MIMAT0000260	UGGUUCUAGACUUGCCAACUA	174
hsa-mir-183	MIMAT0000261	UAUGGCACUGGUAGAAUUCACU	175
hsa-mir-183*	MIMAT0004560	GUGAAUUAACCGAAGGGCCAUA	176
hsa-mir-185	MIMAT0000455	UGGAGAGAAAGGCAGUUCCUGA	177
hsa-mir-185*	MIMAT0004611	AGGGGCUGGCUUUCCUCUGGUC	178
hsa-mir-186	MIMAT0000456	CAAAGAAUUCUCCUUUUGGGCU	179

hsa-mir-186*	MIMAT0004612	GCCCCAAAGGUGAAUUUUUUGGG	180
hsa-mir-190	MIMAT0000458	UGAUUAUGUUUGAUAAUAUAGGU	181
hsa-mir-191	MIMAT0000440	CAACGGAAUCCCCAAAAGCAGCUG	182
hsa-mir-191*	MIMAT0001618	GCUGCGCUUGGAUUUCGUCCCC	183
hsa-mir-192	MIMAT0000222	CUGACCUAUGAAUUGACAGCC	184
hsa-mir-192*	MIMAT0004543	CUGCCAAUUCCAUAGGUCACAG	185
hsa-mir-193a-3p	MIMAT0000459	AACUGGGCCUACAAAGUCCCAGU	186
hsa-mir-193a-5p	MIMAT0004614	UGGGUCUUUGCGGGCGAGAUGA	187
hsa-mir-193b	MIMAT0002819	AACUGGGCCCUCAAAGUCCCAGU	188
hsa-mir-193b*	MIMAT0004767	CGGGGUUUUGAGGGCGAGAUGA	189
hsa-mir-195	MIMAT0000461	UAGCAGCACAGAAAUAUUGGC	190
hsa-mir-195*	MIMAT0004615	CCAAUAUUGGCUGUGCUGCUCC	191
hsa-mir-196a*	MIMAT0004562	CGGCAACAAGAAACUGCCUGAG	192
hsa-mir-196a-1	MIMAT0000226	UAGGUAGUUUCAUGUUGUJUGGG	193
hsa-mir-196a-2	MIMAT0000226	UAGGUAGUUUCAUGUUGUJUGGG	194
hsa-mir-196b	MIMAT0001080	UAGGUAGUUUCCUGUUGUJUGGG	195
hsa-mir-197	MIMAT0000227	UUCACCACCUUCUCCACCCAGC	196
hsa-mir-198	MIMAT0000228	GGUCCAGAGGGGAGAUAGGUUC	197
hsa-mir-199a-3p	MIMAT0000232	ACAGUAGUCUGCACAUUGGUUA	198
hsa-mir-199a-5p	MIMAT0000231	CCCAGUGUUCAGACUACCUGUUC	199
hsa-mir-199a-5p	MIMAT0000231	CCCAGUGUUCAGACUACCUGUUC	200
hsa-mir-199b-3p	MIMAT0004563	ACAGUAGUCUGCACAUUGGUUA	201
hsa-mir-199b-5p	MIMAT0000263	CCCAGUGUUUAGACUAUCUGUUC	202
hsa-mir-200a	MIMAT0000682	UAACACUGUCUGGUACAGAUGU	203
hsa-mir-200a*	MIMAT0001620	CAUCUUACCGGACAGUGCUGGA	204
hsa-mir-200b	MIMAT0000318	UAAAACUGCCUGGUAAUGAUGA	205
hsa-mir-200b*	MIMAT0004571	CAUCUUACUGGGCAGCAUUGGA	206
hsa-mir-200c	MIMAT0000617	UAAAACUGCCGGGUAAUGAUGGA	207
hsa-mir-200c*	MIMAT0004657	CGUCUUACCCAGCAGUGUUUGG	208
hsa-mir-203	MIMAT0000264	GUGAAAUGUUUAGGACCACUAG	209
hsa-mir-204	MIMAT0000265	UUCCCCUUUGUCAUCCUAUGCCU	210
hsa-mir-205	MIMAT0000266	UCCUUCAUCCACCGGAGUCUG	211
hsa-mir-210	MIMAT0000267	CUGUGCGUGUGACAGCGGCUGA	212
hsa-mir-213	MIMAT0000256	AAACAUCAACGCUGUCGGUGAGU	213
hsa-mir-214	MIMAT0000271	ACAGCAGGCACAGACAGGCAGU	214
hsa-mir-214*	MIMAT0004564	UGCCUGUCUACACUUGCUGUGC	215
hsa-mir-216a	MIMAT0000273	UAAUCUCAGCUGGCAACUGUGA	216
hsa-mir-216b	MIMAT0004959	AAAUCUCUGCAGGCAAUGUGA	217
hsa-mir-217	MIMAT0000274	UACUGCAUCAGGAACUGAUUGGA	218
hsa-mir-218-1	MIMAT0000275	UUGUGCUUGAUCUAACCAUGU	219
hsa-mir-218-1*	MIMAT0004565	AUGGUUCCGUCAAGCACCAUGG	220
hsa-mir-218-2	MIMAT0000275	UUGUGCUUGAUCUAACCAUGU	221
hsa-mir-218-2*	MIMAT0004566	CAUGGUUCUGUCAAGCACCGCG	222
hsa-mir-221	MIMAT0000278	AGCUACAUUGUCUGCUGGGUUUC	223
hsa-mir-221*	MIMAT0004568	ACCUGGCAUACAAUGUAGAUUU	224
hsa-mir-222	MIMAT0000279	AGCUACAUUCUGGUACUGGGU	225

hsa-mir-222*	MIMAT0004569	CUCAGUAGCCAGUGUAGAUCCU	226
hsa-mir-223	MIMAT0000280	UGUCAGUUUGUCAAAUACCCCA	227
hsa-mir-223*	MIMAT0004570	CGUGUAUUUGACAAGCUGAGUU	228
hsa-mir-224	MIMAT0000281	CAAGUCACUAGUGGUUCCGUU	229
hsa-mir-302a	MIMAT0000684	UAAGUGCUUCCAUGUUUUGGUGA	230
hsa-mir-302a*	MIMAT0000683	ACUUAACGUGGAUGUACUUGCU	231
hsa-mir-302b	MIMAT0000715	UAAGUGCUUCCAUGUUUAGUAG	232
hsa-mir-302b*	MIMAT0000714	ACUUUAACAUGGAAGUGCUUUC	233
hsa-mir-302c	MIMAT0000717	UAAGUGCUUCCAUGUUUCAGUGG	234
hsa-mir-302c*	MIMAT0000716	UUUAACAAUGGGGUACCUGCUG	235
hsa-mir-302d	MIMAT0000718	UAAGUGCUUCCAUGUUUGAGUGU	236
hsa-mir-302d*	MIMAT0004685	ACUUUAACAUGGAGGCACUUGC	237
hsa-mir-302e	MIMAT0005931	UAAGUGCUUCCAUGCUU	238
hsa-mir-302f	MIMAT0005932	UAUUGCUUCCAUGUUU	239
hsa-mir-320a	MIMAT0000510	AAAAGCUGGGUUGAGAGGGCGA	240
hsa-mir-320b-1	MIMAT0005792	AAAAGCUGGGUUGAGAGGGCAA	241
hsa-mir-320b-2	MIMAT0005792	AAAAGCUGGGUUGAGAGGGCAA	242
hsa-mir-320c-1	MIMAT0005793	AAAAGCUGGGUUGAGAGGGU	243
hsa-mir-320c-2	MIMAT0005793	AAAAGCUGGGUUGAGAGGGU	244
hsa-mir-320d-1	MIMAT0006764	AAAAGCUGGGUUGAGAGGA	245
hsa-mir-320d-2	MIMAT0006764	AAAAGCUGGGUUGAGAGGA	246
hsa-mir-324-3p	MIMAT0000762	ACUGCCCCAGGUGCUGCUUG	247
hsa-mir-324-5p	MIMAT0000761	CGCAUCCCCUAGGGCAUUGGUGU	248
hsa-mir-326	MIMAT0000756	CCUCUGGGCCCUCUCCAG	249
hsa-mir-328	MIMAT0000752	CUGGCCUCUCUGCCCUUCCGU	250
hsa-mir-330-3p	MIMAT0000751	GCAAAGCACACGGCCUGCAGAGA	251
hsa-mir-330-5p	MIMAT0004693	UCUCUGGGCCUGUGCUUAGGC	252
hsa-mir-331-3p	MIMAT0000760	GCCCCUGGGCCUAUCCUAGAA	253
hsa-mir-331-5p	MIMAT0004700	CUAGGUAUUGGUCCCAGGGAUCC	254
hsa-mir-335	MIMAT0000765	UCAAGAGCAAUAACGAAAAAUGU	255
hsa-mir-335*	MIMAT0004703	UUUUUCAUUAUUGCUCCUGACC	256
hsa-mir-339-3p	MIMAT0004702	UGAGCGCCUCGACGACAGAGCCG	257
hsa-mir-339-5p	MIMAT0000764	UCCCUGUCCUCCAGGAGCUCACG	258
hsa-mir-340	MIMAT0004692	UUUAUAAGCAAUGAGACUGAUU	259
hsa-mir-340*	MIMAT0000750	UCCGUCUCAGUUACUUUAUAGC	260
hsa-mir-342-3p	MIMAT0000753	UCUCACACAGAAAUCGCACCCGU	261
hsa-mir-342-5p	MIMAT0004694	AGGGGUGCUAUCUGUGAUUGA	262
hsa-mir-345	MIMAT0000772	GCUGACUCCUAGUCCAGGGCUC	263
hsa-mir-361-3p	MIMAT0004682	UCCCCCAGGUGUGAUUCUGAUUU	264
hsa-mir-361-5p	MIMAT0000703	UUAUCAGAAUCUCCAGGGUAC	265
hsa-mir-370	MIMAT0000722	GCCUGCUGGGUGGAACCUGGU	266
hsa-mir-374a	MIMAT0000727	UUUAUAUACAACCUGUAAGUG	267
hsa-mir-374b	MIMAT0004955	AUAAUAUACAACCUGCUAAGUG	268
hsa-mir-376a*	MIMAT0003386	GUAGAUUCUCCUUCUAUGAGUA	269
hsa-mir-376a-1	MIMAT0000729	AUCAUAGAGGAAAAUCCACGU	270
hsa-mir-376a-2	MIMAT0000729	AUCAUAGAGGAAAAUCCACGU	271

hsa-mir-376b	MIMAT0002172	AUCAUAGAGGAAAAUCCAUGUU	272
hsa-mir-376c	MIMAT0000720	AACAUAGAGGAAAUCCACGU	273
hsa-mir-378	MIMAT0000732	ACUGGACUUGGAGUCAGAAGG	274
hsa-mir-378*	MIMAT0000731	CUCCUGACUCCAGGUCCUGUGU	275
hsa-mir-382	MIMAT0000737	GAAGUUGUUCGUGGGUGGAUCG	276
hsa-mir-411	MIMAT0003329	UAGUAGACCGUUAUGCGUACG	277
hsa-mir-411*	MIMAT0004813	UAUGUAACACGGGUCCACUAACC	278
hsa-mir-423	MIMAT0004748	UGAGGGGCAGAGAGCGAGACUUU	279
hsa-mir-423*	MIMAT0001340	AGCUCGGUCUGAGGCCCUACGU	280
hsa-mir-425-3p	MIMAT0001343	AUCGGGAAUGUCGUGGUCCGCC	281
hsa-mir-425-5p	MIMAT0003393	AAUGACACGAUCACUCCCGUUGA	282
hsa-mir-432	MIMAT0002814	UCUUGGAGUAGGUCAUUGGGUGG	283
hsa-mir-432*	MIMAT0002815	CUGGAUGGCUCCCUCCAUUGUCU	284
hsa-mir-433	MIMAT0001627	AUCAUGAUGGGCUCCUCGGUGU	285
hsa-mir-484	MIMAT0002174	UCAGGCUCAGUCCCCUCCCGAU	286
hsa-mir-485-3p	MIMAT0002176	GUCAUACACGGCUCUCCUCU	287
hsa-mir-485-5p	MIMAT0002175	AGAGGCUGGCCGUGAUGAAUUC	288
hsa-mir-486-3p	MIMAT0004762	CGGGGCAGCUCAGUACAGGAU	289
hsa-mir-486-5p	MIMAT0002177	UCCUGUACUGAGCUGCCCCGAG	290
hsa-mir-487a	MIMAT0002178	AAUCAUACAGGGACAUCCAGUU	291
hsa-mir-487b	MIMAT0003180	AAUCGUACAGGGUCAUCCACUU	292
hsa-mir-532	MIMAT0002888	CAUGCCUUGAGUGUAGGACCGU	293
hsa-mir-532-5p	MIMAT0004780	CCUCCCACACCCAAGGCUUGCA	294
hsa-mir-539	MIMAT0003163	GGAGAAAUAUCCUUGGUGUGU	295
hsa-mir-574-3p	MIMAT0003239	CACGCUCUAUGCACACACCCACA	296
hsa-mir-574-5p	MIMAT0004795	UGAGUGUGUGUGUGAGUGUGU	297
hsa-mir-584	MIMAT0003249	UUAUGGUUUGCCUGGGACUGAG	298
hsa-mir-628-3p	MIMAT0003297	UCUAGUAAGAGUGGGCAGUCGA	299
hsa-mir-628-5p	MIMAT0004809	AUGCUGACAUAAUUAUCUAGAGG	300
hsa-mir-643	MIMAT0003313	ACUUGUAUGCUAGCUCAGGUAG	301
hsa-mir-660	MIMAT0003338	UACCCAUUGCAUAUCGGAGUUG	302

## [00153] References

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**CLAIMS:**

1. A method of diagnosing or prognosticating prostate cancer in a subject, comprising:
  - i) isolating microvesicles from a peripheral blood sample of the subject;
  - ii) determining the level of at least one miR gene product in the isolated microvesicles; and
  - iii) comparing the level of the at least one miR gene product in the sample to a control, wherein an increase in the level of the at least one miR gene product in the sample from the subject, relative to that of the control, is diagnostic or prognostic of the prostate cancer; wherein the level of miR-21 and at least one of the following MiRs is determined: miR-15a, miR-16-1, miR-143 and miR-145.
2. Use of isolated microvesicles as a biomarker for prostate cancer, wherein the biomarker is isolated from microvesicles in peripheral blood of a subject having the prostate cancer, and at least the following MiRs are upregulated in the isolated microvesicle relative to that of the control subject: miR-21, and at least the following miRs are down regulated in the isolated microvesicle relative to that of the control subject: miR-15a, miR-16-1, miR-143 and miR-145.
3. The method of claim 1, wherein the control is selected from the group consisting of:
  - i) a reference standard;
  - ii) the level of the at least one miR gene product in isolated microvesicles from a subject that does not have the disorder; and
  - iii) the level of the at least one miR gene product in isolated microvesicles from a sample of the subject that does not exhibit such disorder.
4. The method of claim 1, wherein the sample from the subject comprises plasma.

5. The method of claim 1 or 4, wherein the level of the at least one miR gene product is measured by RT-PCR, Northern blot analysis, solution hybridization detection, and/or microarray analysis.

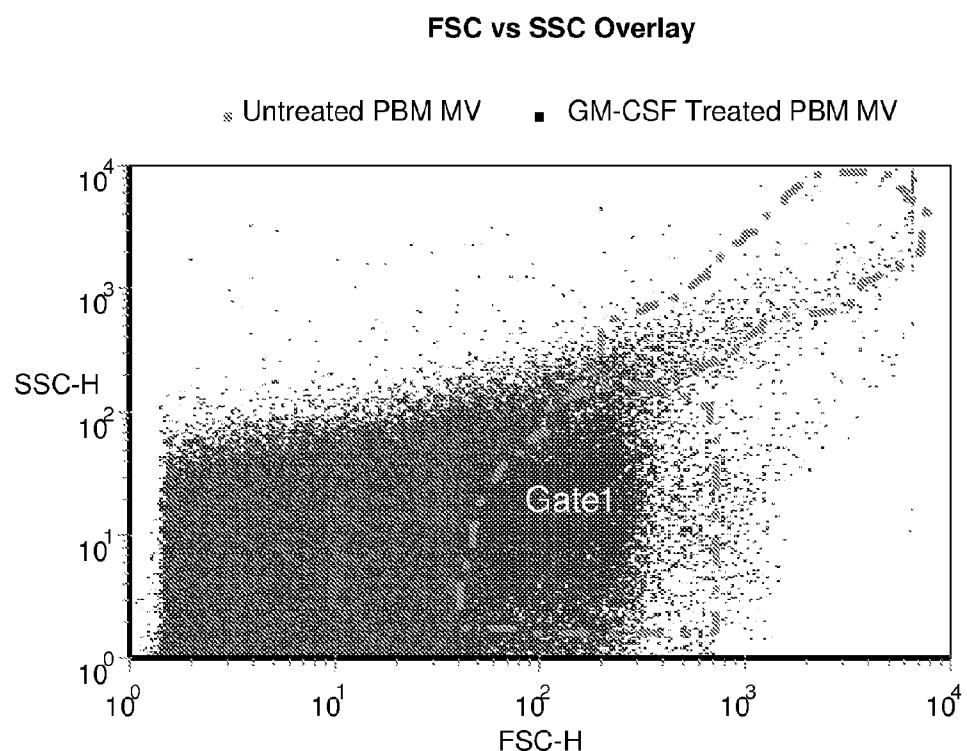
6. The method of claim 1, 4 or 5, wherein isolating microvesicles comprises centrifugation.

7. The method of claim 1, 4, 5 or 6, wherein miR-21 is upregulated in the isolated microvesicle relative to that of the control subject; and at least one of the following miRs are down regulated in the isolated microvesicle relative to that of the control subject: miR-15a, miR16-1, miR-143 and miR-145.

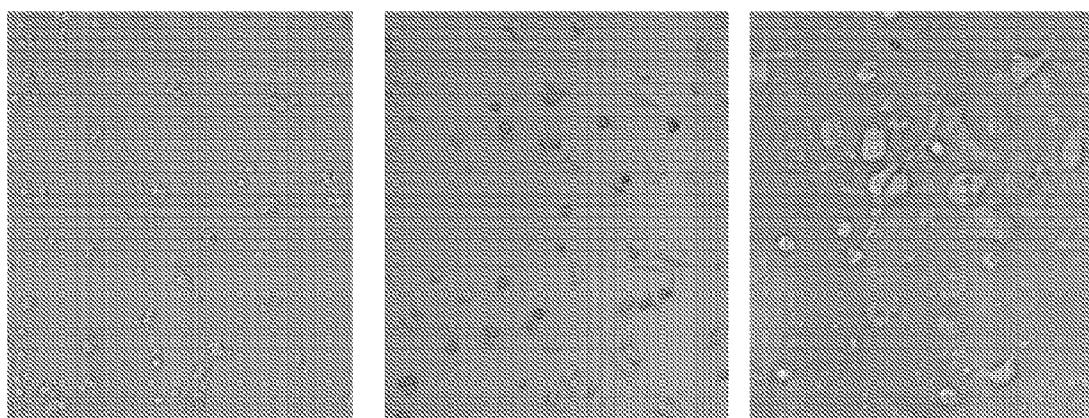
**The Ohio State University Research Foundation**

**Patent Attorneys for the Applicant/Nominated Person**

**SPRUSON & FERGUSON**



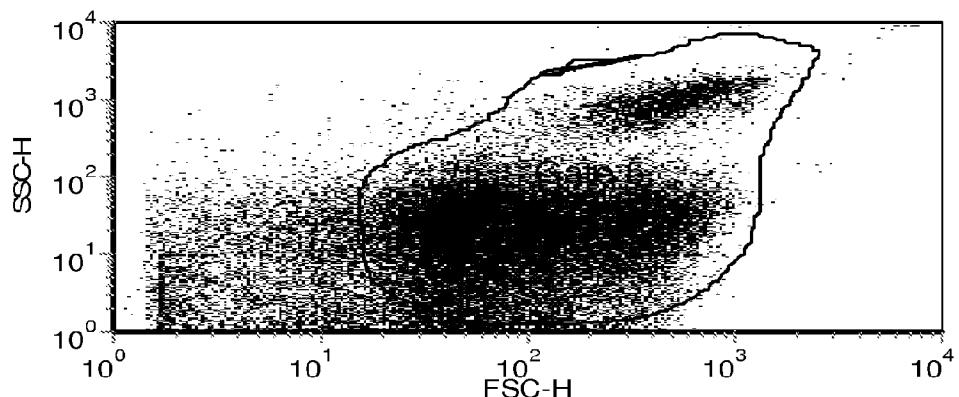
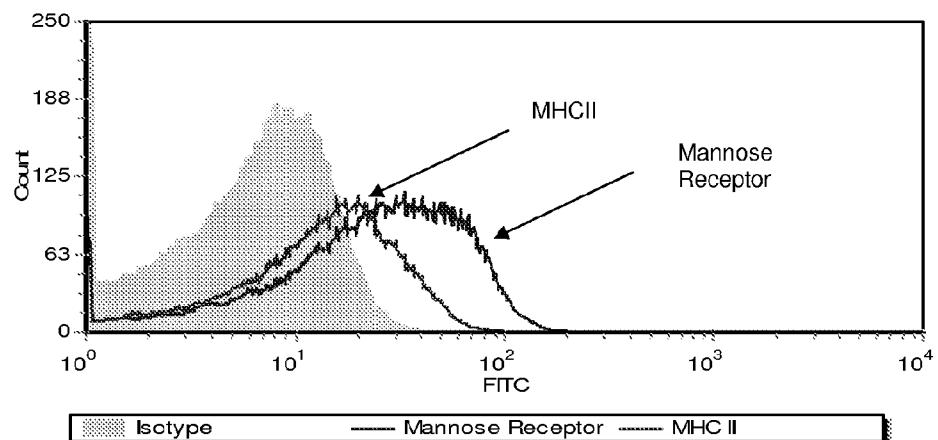
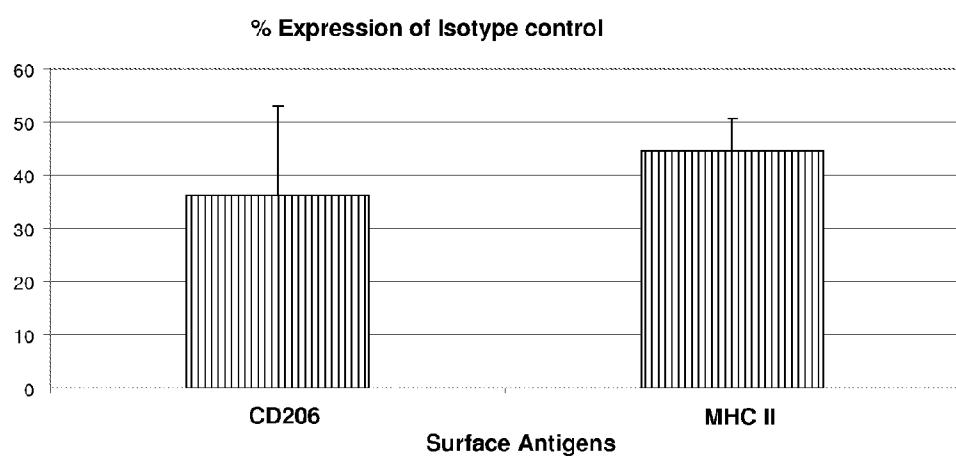
**Figure 1**

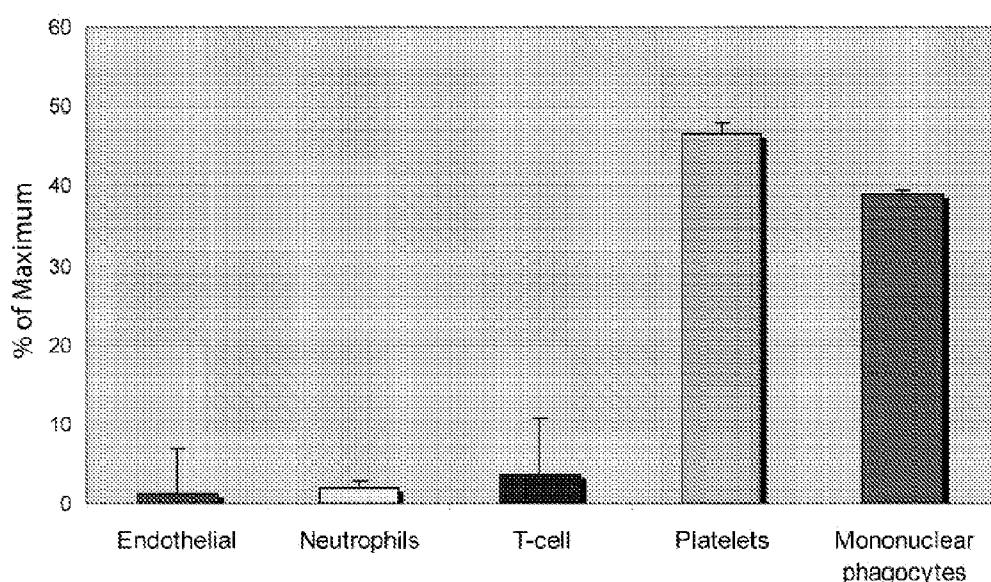


**Fig. 2a**

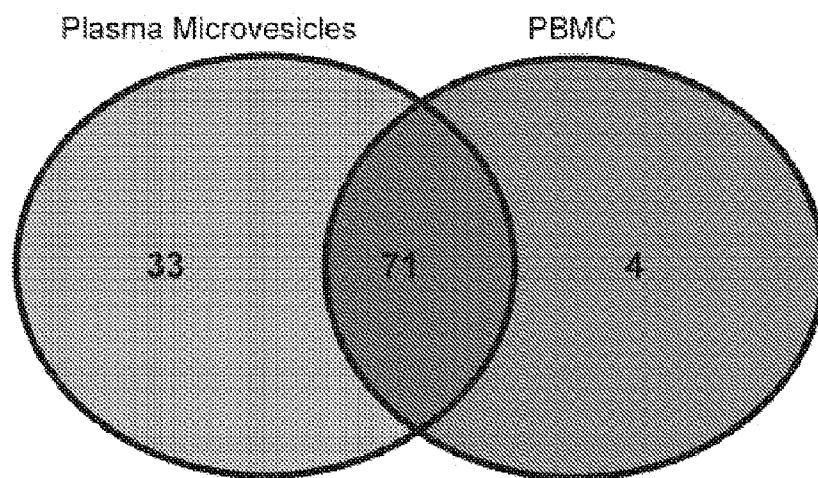
**Fig. 2b**

**Fig. 2c**

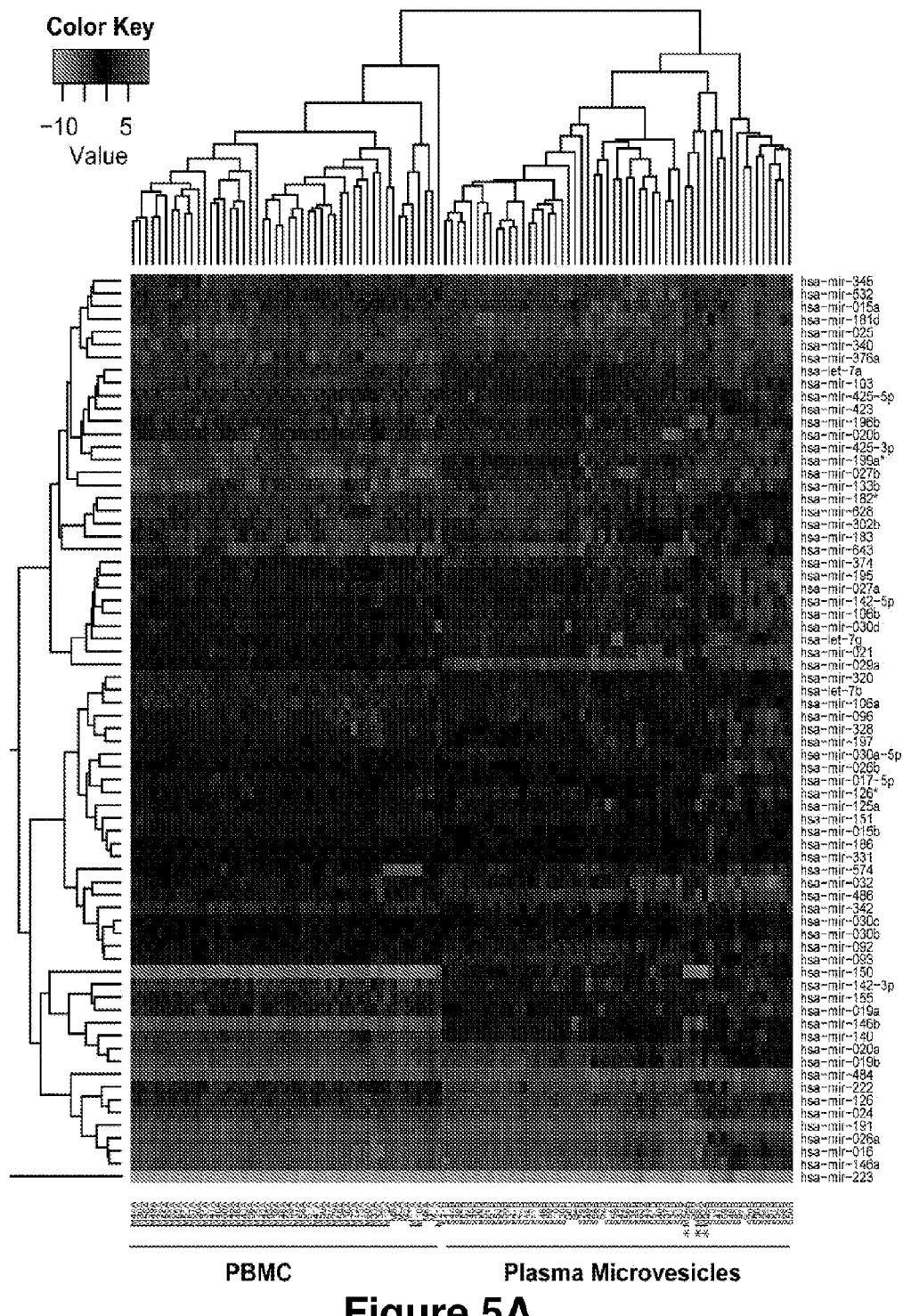
**Figure 3a****Figure 3b****Figure 3c**



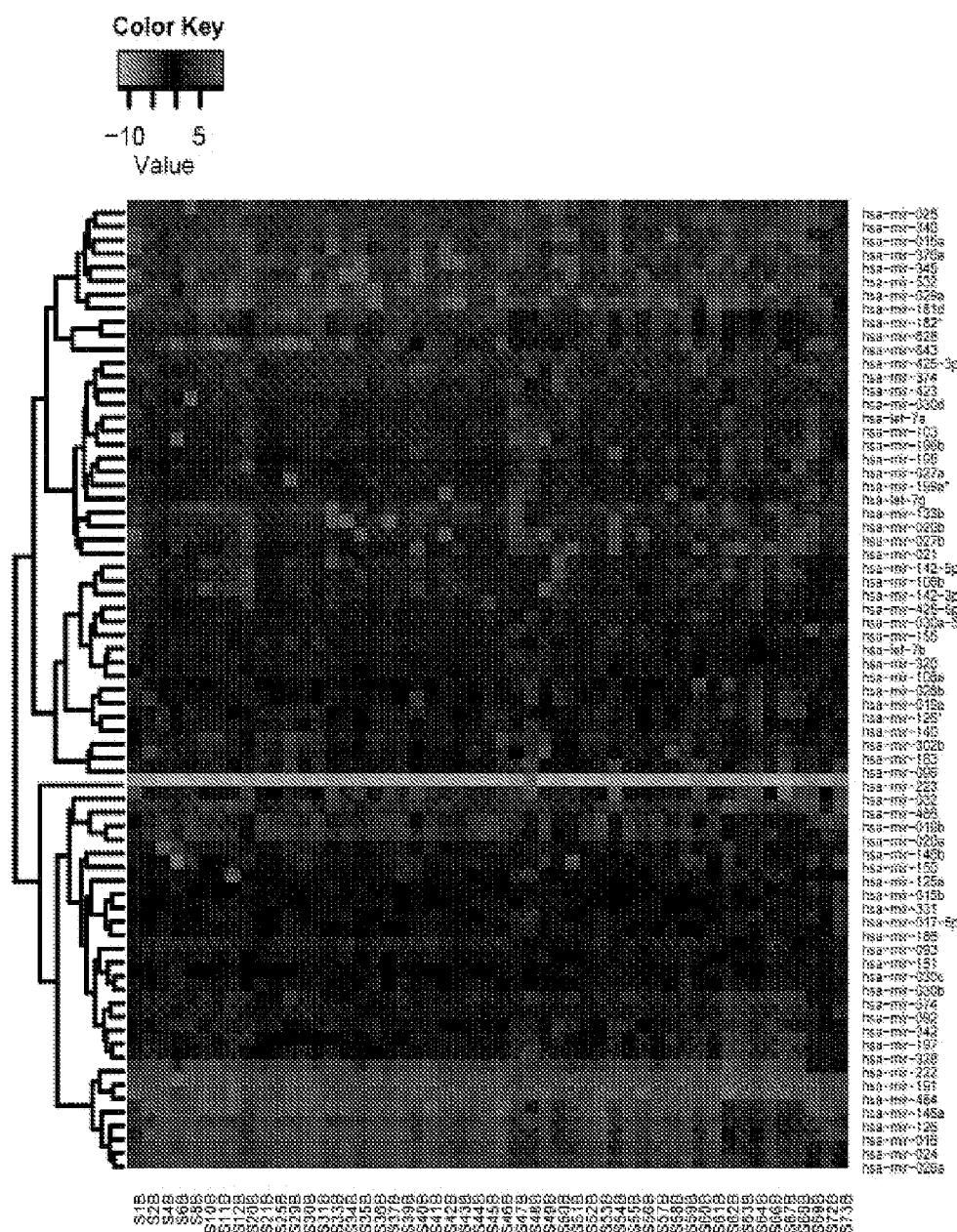
**Figure 4**

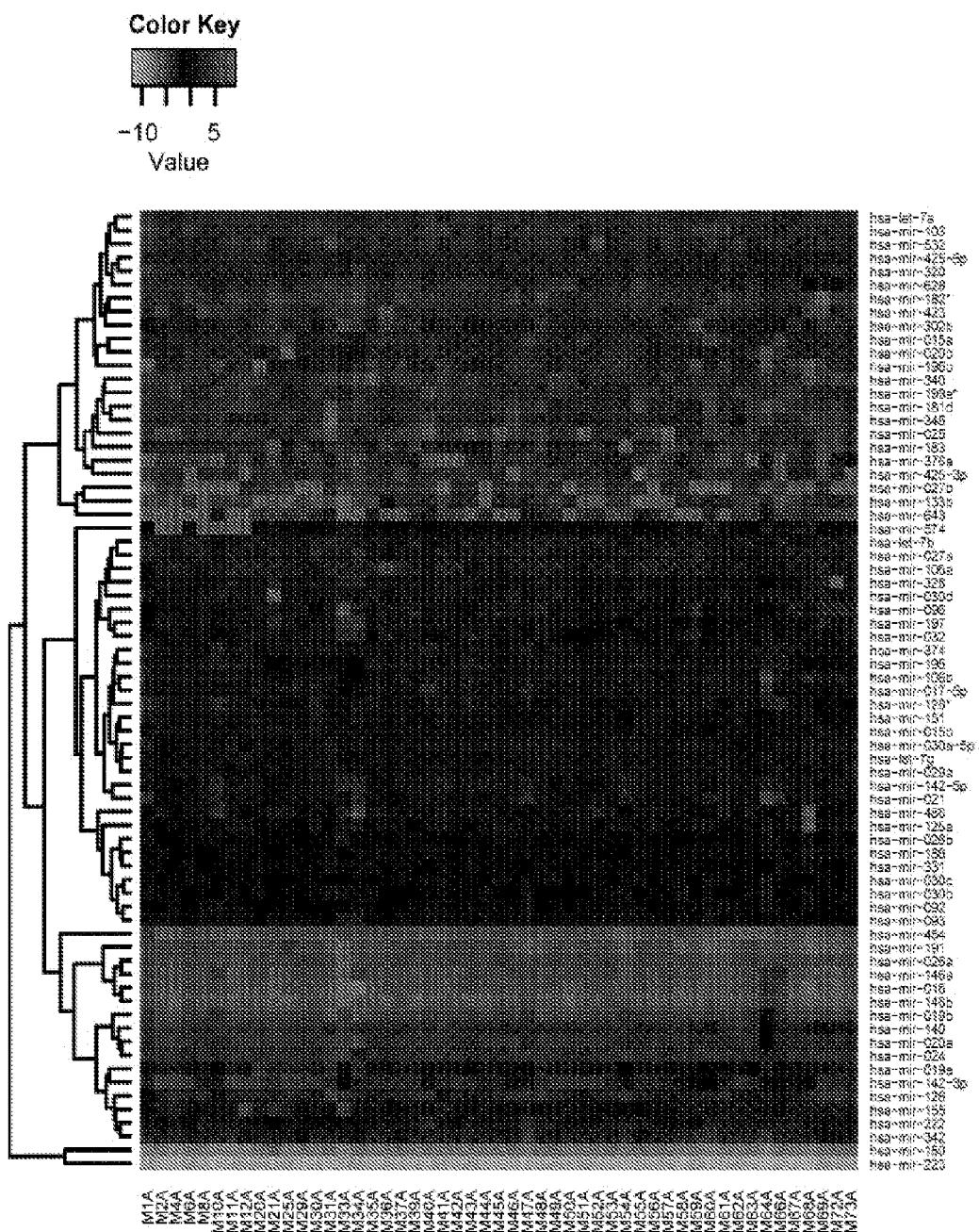


**Figure 5D**



**Figure 5A**

**Figure 5B**



**Figure 5C**

Table I

Disease	Increased Expression in Disease Tissue	Decreased Expression in Disease Tissue	Reference
colon adenocarcinoma	miR-20a, miR-21, miR-106a, miR-181b, <b>miR-203</b>		JAMA. 2008 Jan 30; 299(4):425-36; Int J Cancer. 2008 Mar 1; 122(5):969-77.
Colorectal	miR-19a, miR-21 miR-127, <b>miR-31</b> , miR-96, <b>miR-135b</b> and miR-183,	<b>miR-30c</b> , miR-133a, miR-143, <b>miR-133b</b> and <b>miR-145</b>	Int J Cancer. 2008 Mar 1; 122(5):969-77; Cancer. 2006 Jul 19; 106(3):529; Braz J Med Biol Res. 2007 Nov; 40(11):1435-40.
Prostate Cancer	miR-21	<b>miR-15a</b> , miR-16-1, miR-143, <b>miR-145</b>	Int J Cancer. 2008 Mar 1; 122(5):969-77.
Lung Cancer	miR-17-92, miR-19a, miR-21, miR-92, miR-155, miR-191, <b>miR-205</b> , miR- <b>210</b>	<b>miR-let-7</b>	Int J Cancer. 2008 Mar 1; 122(5):969-77.
Breast cancer	miR-21, miR-155	miR-125b, <b>miR-145</b>	Int J Cancer. 2008 Mar 1; 122(5):969-77.
B-Cell lymphoma	miR-155, miR-17-92, miR-19a, miR-92, miR-142, miR-155, miR-221		Int J Cancer. 2008 Mar 1; 122(5):969-77.
Pancreatic	miR-103 and <b>miR-107</b> , miR-18a, <b>miR-31</b> , miR-93, miR-221 and miR-224, miR-155	miR-133a, miR-216, miR 217	J Clin Oncol. 2006 Oct 10; 24(29):4677-84; Oncogene. 2007 Jun 28; 26(30):4442-52
Diffuse large BCL	miR-155, miR-17-92		Int J Cancer. 2008 Mar 1; 122(5):969-77.
CLL	<b>miR-23b</b> , miR-24-1, miR-146, miR-155, miR-195, miR-221, miR-331, miR-29a, miR-195, <b>miR-34a</b> , and <b>miR-29c</b>	<b>miR-15a</b> , <b>miR-16-1</b> , miR-29, miR-223	Int J Cancer. 2008 Mar 1; 122(5):969-77; Braz J Med Biol Res. 2007 Nov; 40(11):1435-40.
Bladder cancer	miR-223, miR-26b, miR-221, miR-103-1, miR-185, miR-23b, <b>miR-203</b> , miR-17-5p, <b>miR-23a</b> , and <b>miR-205</b>		Urol Oncol. 2007 Sep-Oct; 25(5):387-92
Renal Cancer	<b>miR-28</b> , <b>miR-185</b> , miR-27, and miR-let-7f-2		Urol Oncol. 2007 Sep-Oct; 25(5):387-92
Hypoxia-tumor	<b>miR-23</b> , miR-24, miR-26, miR-27, miR-103, <b>miR-107</b> , <b>miR-181</b> , <b>miR-210</b> , and miR-213		Mol Cell Biol. 2007 Mar; 27(5):1859-67.
uterine leiomyomas	miR-let-7 family, miR-21, <b>miR-23b</b> , <b>miR-29b</b> , and miR-197		Genes Chromosomes Cancer. 2007 Apr; 46(4):336-47
ovarian	miR-199*, <b>miR-200a</b> , <b>miR-214</b>	miR-100, <b>miR-let-7 cluster</b> , miR-125b	Cancer Res. 2008 Jan 15; 68(2):425-33
hepatitis C virus-associated hepatocellular carcinoma.	<b>miR-122</b> , <b>miR-100</b> , and <b>miR-10a</b>	miR-198 and <b>miR-145</b>	Hepatology. 2007 Dec 19 [Epub ahead of print]
ALL	<b>miR-128b</b> , <b>miR-204</b> , <b>miR-218</b> , miR-331, and miR-181b-1, miR-17-92		Braz J Med Biol Res. 2007 Nov; 40(11):1435-40.
Alzheimer's disease	<b>miR-9</b> , <b>miR-128</b>	miR-107	J Neurosci. 2008 Jan 30; 28(5):1213-23 Neuroreport. 2007 Feb 12; 18(3):297-300
myelofibrosis	<b>miR-190</b>	miR-31, <b>miR-150</b> , and miR-95	Exp Hematol. 2007 Nov; 35(11):1708-18
myelofibrosis, polycythemia vera, thrombocythemia		miR-34a, <b>-342</b> , -326, -105, -149, and -147	Exp Hematol. 2007 Nov; 35(11):1708-18
HIV	miR-29a, <b>miR-29b</b> , <b>miR-149</b> , <b>miR-378</b> , miR-324-5p		Biochem Biophys Res Commun. 2005 Dec 2; 337(4):1214-8.
HIV-1 latency	<b>miR-28</b> , <b>miR-125b</b> , miR-150, miR-223 and miR-382		Nat Med. 2007 Oct; 13(10):1241-7.

Figure 6 – Table I

Table II

Expressed Plasma miRNAs after filtering		Detector Name	Undetectable		
Detector_Name	filter				
hsa-let-7a	0.333333333	hsa-let-7e	hsa-mir-346	hsa-mir-520c	hsa-mir-629
hsa-let-7b	0.058823529	hsa-mir-001	hsa-mir-362	hsa-mir-520d	hsa-mir-630
hsa-let-7c	0.725490196	hsa-mir-007	hsa-mir-363	hsa-mir-520d*	hsa-mir-631
hsa-let-7d	0.529411765	hsa-mir-009	hsa-mir-363*	hsa-mir-520e	hsa-mir-632
hsa-let-7f	0.745098039	hsa-mir-009*	hsa-mir-365	hsa-mir-520f	hsa-mir-633
hsa-let-7g	0.215686275	hsa-mir-010a	hsa-mir-367	hsa-mir-520g	hsa-mir-634
hsa-mir-015a	0.568627451	hsa-mir-010b	hsa-mir-368	hsa-mir-520h	hsa-mir-635
hsa-mir-015b	0.078431373	hsa-mir-017-3p	hsa-mir-369-3p	hsa-mir-521	hsa-mir-636
hsa-mir-016	0	hsa-mir-369-5p	hsa-mir-522	hsa-mir-637	
hsa-mir-017-5p	0.117647059	hsa-mir-018b	hsa-mir-371	hsa-mir-523	hsa-mir-638
hsa-mir-018a	0.784313726	hsa-mir-022	hsa-mir-372	hsa-mir-524	hsa-mir-639
hsa-mir-018a*	0.784313726	hsa-mir-023a	hsa-mir-373	hsa-mir-525	hsa-mir-641
hsa-mir-019a	0.176470588	hsa-mir-023b	hsa-mir-373*	hsa-mir-525*	hsa-mir-642
hsa-mir-019b	0.058823529	hsa-mir-028	hsa-mir-375	hsa-mir-526a	
hsa-mir-020a	0	hsa-mir-029b	hsa-mir-376a*	hsa-mir-526b	hsa-mir-644
hsa-mir-020b	0.37254902	hsa-mir-029c	hsa-mir-376b	hsa-mir-526b*	hsa-mir-645
hsa-mir-021	0.411764706	hsa-mir-031	hsa-mir-377	hsa-mir-526c	hsa-mir-646
hsa-mir-024	0	hsa-mir-033	hsa-mir-378	hsa-mir-527	hsa-mir-647
hsa-mir-025	0.647058824	hsa-mir-034a	hsa-mir-379		hsa-mir-648
hsa-mir-026a	0	hsa-mir-034b	hsa-mir-380-3p		hsa-mir-649
hsa-mir-026b	0.176470588	hsa-mir-034c	hsa-mir-380-5p	hsa-mir-542-3p	hsa-mir-650
hsa-mir-027a	0.254901961	hsa-mir-095	hsa-mir-381	hsa-mir-542-5p	hsa-mir-651
hsa-mir-027b	0.352941177	hsa-mir-099a	hsa-mir-383	hsa-mir-544	hsa-mir-652
hsa-mir-029a	0.764705882	hsa-mir-100	hsa-mir-409-5p	hsa-mir-545	hsa-mir-653
hsa-mir-030a-3p	0.37254902	hsa-mir-101	hsa-mir-410	hsa-mir-548a	hsa-mir-654
hsa-mir-030a-5p	0.098039216	hsa-mir-105	hsa-mir-412	hsa-mir-548b	hsa-mir-655
hsa-mir-030b	0.039215686	hsa-mir-107	hsa-mir-422a	hsa-mir-548c	hsa-mir-656
hsa-mir-030c	0.019607843	hsa-mir-122a	hsa-mir-422b	hsa-mir-548d	hsa-mir-657
hsa-mir-030d	0.274509804	hsa-mir-124a	hsa-mir-424	hsa-mir-549	hsa-mir-658
hsa-mir-032	0	hsa-mir-125b	hsa-mir-429	hsa-mir-550	hsa-mir-659
hsa-mir-092	0	hsa-mir-126	hsa-mir-432*	hsa-mir-551a	hsa-mir-660
hsa-mir-093	0.058823529	hsa-mir-128a	hsa-mir-448	hsa-mir-551b	hsa-mir-661
hsa-mir-096	0	hsa-mir-128b	hsa-mir-449	hsa-mir-552	hsa-mir-662
hsa-mir-098	0.784313726	hsa-mir-129	hsa-mir-449b	hsa-mir-553	
hsa-mir-099b	0.549019608	hsa-mir-133a	hsa-mir-450	hsa-mir-554	

Figure 7 – Table II

hsa-mir-103	0.235294118	<b>hsa-mir-135a</b>	<b>hsa-mir-451</b>	<b>hsa-mir-555</b>
hsa-mir-106a	0.058823529	<b>hsa-mir-135b</b>	<b>hsa-mir-452</b>	<b>hsa-mir-556</b>
hsa-mir-106b	0.215686275	<b>hsa-mir-136</b>	<b>hsa-mir-452*</b>	<b>hsa-mir-557</b>
hsa-mir-125a	0.078431373	<b>hsa-mir-137</b>	<b>hsa-mir-453</b>	<b>hsa-mir-558</b>
hsa-mir-126	0	<b>hsa-mir-138</b>	<b>hsa-mir-455</b>	<b>hsa-mir-559</b>
hsa-mir-126*	0.117647059	<b>hsa-mir-139</b>	<b>hsa-mir-483</b>	<b>hsa-mir-561</b>
hsa-mir-127	0.176470588	<b>hsa-mir-141</b>	<b>hsa-mir-485-5p</b>	<b>hsa-mir-562</b>
hsa-mir-130a	0.666666667	<b>hsa-mir-143</b>	<b>hsa-mir-487a</b>	<b>hsa-mir-563</b>
hsa-mir-130b	0.705882353	<b>hsa-mir-147</b>	<b>hsa-mir-488</b>	<b>hsa-mir-564</b>
hsa-mir-132	0.705882353	<b>hsa-mir-148a</b>	<b>hsa-mir-489</b>	<b>hsa-mir-565</b>
hsa-mir-133b	0.529411765	<b>hsa-mir-149</b>	<b>hsa-mir-491</b>	<b>hsa-mir-566</b>
hsa-mir-134	0.235294118	<b>hsa-mir-152</b>	<b>hsa-mir-492</b>	<b>hsa-mir-567</b>
hsa-mir-140	0.078431373	<b>hsa-mir-153</b>	<b>hsa-mir-493-3p</b>	<b>hsa-mir-569</b>
hsa-mir-142-3p	0.196078431	<b>hsa-mir-154</b>	<b>hsa-mir-493-5p</b>	<b>hsa-mir-570</b>
hsa-mir-142-5p	0.254901961	<b>hsa-mir-154*</b>	<b>hsa-mir-494</b>	<b>hsa-mir-571</b>
hsa-mir-145	0.705882353	<b>hsa-mir-181a</b> <b>hsa-mir-181a*</b>	<b>hsa-mir-495</b>	<b>hsa-mir-572</b>
hsa-mir-146a	0	<b>hsa-mir-181a*</b>	<b>hsa-mir-496</b>	<b>hsa-mir-573</b>
hsa-mir-146b	0.078431373	<b>hsa-mir-181c</b>	<b>hsa-mir-497</b>	
hsa-mir-148b	0.784313726	<b>hsa-mir-182</b>	<b>hsa-mir-498</b>	<b>hsa-mir-575</b>
hsa-mir-150	0.019607843	<b>hsa-mir-184</b>	<b>hsa-mir-499</b>	<b>hsa-mir-576</b>
hsa-mir-151	0.019607843	<b>hsa-mir-185</b>	<b>hsa-mir-500</b>	<b>hsa-mir-578</b>
hsa-mir-155	0.176470588	<b>hsa-mir-187</b>	<b>hsa-mir-501</b>	<b>hsa-mir-579</b>
hsa-mir-181d	0.725490196	<b>hsa-mir-189</b>	<b>hsa-mir-502</b>	<b>hsa-mir-580</b>
hsa-mir-182*	0.196078431	<b>hsa-mir-190</b>	<b>hsa-mir-503</b>	<b>hsa-mir-583</b>
hsa-mir-183	0.058823529	<b>hsa-mir-192</b>	<b>hsa-mir-504</b>	
hsa-mir-186	0.039215686	<b>hsa-mir-193b</b>	<b>hsa-mir-505</b>	<b>hsa-mir-585</b>
hsa-mir-191	0	<b>hsa-mir-194</b>	<b>hsa-mir-506</b>	<b>hsa-mir-586</b>
hsa-mir-193a	0.666666667	<b>hsa-mir-196a</b>	<b>hsa-mir-507</b>	<b>hsa-mir-587</b>
hsa-mir-195	0.31372549	<b>hsa-mir-198</b>	<b>hsa-mir-508</b>	<b>hsa-mir-588</b>
hsa-mir-196b	0.254901961	<b>hsa-mir-199a</b>	<b>hsa-mir-509</b>	<b>hsa-mir-589</b>
hsa-mir-197	0.019607843	<b>hsa-mir-199b</b>	<b>hsa-mir-510</b>	<b>hsa-mir-591</b>
hsa-mir-199a*	0.254901961	<b>hsa-mir-200a</b> <b>hsa-mir-200a*</b>	<b>hsa-mir-511</b>	<b>hsa-mir-592</b>
hsa-mir-221	0.784313726	<b>hsa-mir-512-3p</b>	<b>hsa-mir-593</b>	
hsa-mir-222	0	<b>hsa-mir-200b</b>	<b>hsa-mir-512-5p</b>	<b>hsa-mir-594</b>
hsa-mir-223	0	<b>hsa-mir-200c</b>	<b>hsa-mir-513</b>	<b>hsa-mir-596</b>
hsa-mir-224	0.529411765	<b>hsa-mir-202</b>	<b>hsa-mir-514</b>	<b>hsa-mir-597</b>
hsa-mir-302b	0.078431373	<b>hsa-mir-202*</b>	<b>hsa-mir-515-3p</b>	<b>hsa-mir-599</b>
hsa-mir-320	0.098039216	<b>hsa-mir-203</b>	<b>hsa-mir-515-5p</b>	<b>hsa-mir-600</b>
hsa-mir-324-3p	0.705882353	<b>hsa-mir-204</b>	<b>hsa-mir-516-5p</b>	<b>hsa-mir-601</b>
hsa-mir-324-5p	0.470588235	<b>hsa-mir-205</b>	<b>hsa-mir-517*</b>	<b>hsa-mir-603</b>
hsa-mir-328	0	<b>hsa-mir-206</b>	<b>hsa-mir-517a</b>	<b>hsa-mir-604</b>

**Figure 7 – Table II (cont)**

hsa-mir-330	0.666666667	<b>hsa-mir-208</b>	<b>hsa-mir-517b</b>	<b>hsa-mir-606</b>
hsa-mir-331	0.039215686	<b>hsa-mir-210</b>	<b>hsa-mir-517c</b>	<b>hsa-mir-607</b>
hsa-mir-335	0.725490196	<b>hsa-mir-211</b>	<b>hsa-mir-518a</b>	<b>hsa-mir-608</b>
hsa-mir-339	0.607843137	<b>hsa-mir-214</b>	<b>hsa-mir-518b</b>	<b>hsa-mir-609</b>
hsa-mir-340	0.529411765	<b>hsa-mir-215</b>	<b>hsa-mir-518c</b>	<b>hsa-mir-610</b>
hsa-mir-342	0.019607843	<b>hsa-mir-216</b>	<b>hsa-mir-518c*</b>	<b>hsa-mir-612</b>
hsa-mir-345	0.705882353	<b>hsa-mir-217</b>	<b>hsa-mir-518d</b>	<b>hsa-mir-613</b>
hsa-mir-361	0.725490196	<b>hsa-mir-218</b>	<b>hsa-mir-518e</b>	<b>hsa-mir-614</b>
hsa-mir-370	0.549019608	<b>hsa-mir-219</b>	<b>hsa-mir-518f</b>	<b>hsa-mir-615</b>
hsa-mir-374	0.37254902	<b>hsa-mir-220</b>	<b>hsa-mir-519a</b>	<b>hsa-mir-616</b>
hsa-mir-376a	0.450980392	<b>hsa-mir-296</b> <b>hsa-mir-299-</b>	<b>hsa-mir-519b</b>	<b>hsa-mir-617</b>
hsa-mir-382	0.607843137	<b>3p</b> <b>hsa-mir-299-</b>	<b>hsa-mir-519c</b>	<b>hsa-mir-618</b>
hsa-mir-411	0.607843137	<b>5p</b>	<b>hsa-mir-519d</b>	<b>hsa-mir-619</b>
hsa-mir-423	0.215686275	<b>hsa-mir-301</b>	<b>hsa-mir-519e</b>	<b>hsa-mir-621</b>
hsa-mir-425-3p	0.31372549	<b>hsa-mir-302a</b> <b>hsa-mir-</b>	<b>hsa-mir-519e*</b>	<b>hsa-mir-622</b>
hsa-mir-425-5p	0.215686275	<b>302a*</b> <b>hsa-mir-</b>	<b>hsa-mir-520a</b>	<b>hsa-mir-624</b>
hsa-mir-432	0.392156863	<b>302b*</b>	<b>hsa-mir-520a*</b>	<b>hsa-mir-626</b>
hsa-mir-433	0.470588235	<b>hsa-mir-302c</b> <b>hsa-mir-</b>	<b>hsa-mir-520b</b>	<b>hsa-mir-627</b>
hsa-mir-484	0	<b>302c*</b>		
hsa-mir-485-3p	0.31372549	<b>hsa-mir-302d</b>		
hsa-mir-486	0	<b>hsa-mir-323</b>		
hsa-mir-487b	0.529411765	<b>hsa-mir-325</b>		
hsa-mir-532	0.62745098	<b>hsa-mir-326</b>		
hsa-mir-539	0.588235294	<b>hsa-mir-329</b>		
hsa-mir-574	0	<b>hsa-mir-337</b>		
hsa-mir-584	0.784313726	<b>hsa-mir-338</b>		
hsa-mir-628	0			
hsa-mir-643	0.568627451			

Figure 7 – Table II (cont)

**Table III. Top ten expressed miRNAs in plasma microvesicles and PBMC.**

Plasma Microvesicles				PBMC			
miRNA	Normalized Expression ( $\pm$ S.D.)	Frequency expressed among donors (%)	Top ten ranking frequency (%)	miRNA	Normalized Expression ( $\pm$ S.D.)	Frequency expressed among donors (%)	Top ten ranking frequency (%)
<b>mir-223</b>	1589 $\pm$ 653	100	100	<b>mir-223</b>	2143 $\pm$ 499	100	100
<b>mir-484</b>	50.9 $\pm$ 22.9	100	96	<b>mir-150</b>	241 $\pm$ 94.6	98	98
<b>mir-191</b>	46.4 $\pm$ 14.9	100	100	<b>mir-146b</b>	57.5 $\pm$ 21.1	100	100
<b>mir-146a</b>	39.5 $\pm$ 19	100	88	<b>mir-016</b>	54.7 $\pm$ 32.9	100	100
<b>mir-016</b>	25.4 $\pm$ 13.3	100	78	<b>mir-484</b>	40.6 $\pm$ 18.8	89	88
<b>mir-026a</b>	25.2 $\pm$ 9.95	100	90	<b>mir-146a</b>	39.6 $\pm$ 13.0	100	98
<b>mir-222</b>	24.5 $\pm$ 12.4	100	76	<b>mir-191</b>	32.4 $\pm$ 15.6	100	94
<b>mir-024</b>	22.7 $\pm$ 10.5	100	80	<b>mir-026a</b>	30 $\pm$ 8.92	100	100
<b>mir-126</b>	18.2 $\pm$ 8.04	100	66	<b>mir-019b</b>	21.7 $\pm$ 7.49	100	80
<b>mir-032</b>	15.3 $\pm$ 32.6	100	31	<b>mir-020a</b>	15 $\pm$ 5.11	100	4

**Figure 8 – Table III**

Predicted pathways regulated by miRNAs expressed in the plasma microvesicles and PBMC fractions based on IPA analysis of only Sanger miRBase predicted targets.			
Plasma Microvesicles	p-value	PBMC	p-value
glycerophospholipid metabolism	3.29E-03	axonal guidance signaling	1.47E-02
inositol phosphate metabolism	5.77E-03	synaptic long-term potentiation	2.07E-02
phospholipid degradation	9.17E-03	estrogen receptor signaling	2.23E-02
alanine and aspartate metabolism	1.96E-02	glycerophospholipid metabolism	2.45E-02
estrogen receptor signaling	2.14E-02	D-glutamine and D-glutamate metabolism	2.78E-02

Predicted pathways regulated by miRNAs expressed in the plasma microvesicles and PBMC fractions from combined targets of TargetScan and Sanger mirBase.			
Plasma Microvesicles	p-value	PBMC	p-value
Antigen Presentation Pathway	1.28E-03	Glycine, Serine, Threonine Metabolism	3.63E-03
Glycerophospholipid Metabolism	9.05E-03	Glycerophospholipid Metabolism	2.38E-02
Glycine, Serine, Threonine Metabolism	1.56E-02	D-Glutamine, D-Glutamate Metabolism	2.54E-02
Natural Killer Cell Signaling	1.57E-02	Glyoxylate, Dicarboxylate Metabolism	4.37E-02

**Figure 9- Table IV**

**plasma\_mean** is the average of the raw Ct across all 51 observations for plasma

**PBMC\_mean** is the average of the raw Ct across all 51 observations for PBMC

"Plasma-PBMC" is the difference of normalized data between plasma and PBMC

Plasma-MNC "+": miR level is higher in PBMC, and "---": miR level is lower in plasma

"foldchange" is  $2^{\Delta Ct}$  the difference. For miR 29a:  $40.3 = 2^{\Delta Ct}(5.33)$ . Which means that the miR level in PBMC is 40 times higher than that in serum

"p-value" was considered as significant if p-value < 0.05/72 = 0.0006

Detector_Name	Plasma_mean	PBMC_mean	Plasma-MNC	foldchange	p-value	Fold Change for Plasma
hsa-mir-029a	37.12771586	31.87566924	5.3333	40.31654208	9.70E-28	0.024803715
18S (CT)	12.71460766	9.451029281	3.3449	10.16050356	9.84E-25	0.098420319
hsa-mir-155	32.77301951	29.64446324	3.2098	9.252222752	6.61E-23	0.108082136
hsa-mir-146b	30.87718855	26.44380422	4.5147	22.85915231	1.81E-22	0.043746154
hsa-mir-142-3p	33.45027482	28.97701498	4.5545	23.49855274	1.91E-22	0.042555812
hsa-mir-222	27.69255453	29.77206378	-1.9982	0.250312111	1.18E-21	3.995012452
hsa-mir-328	30.15998382	32.9642692	-2.723	0.151459083	1.64E-21	6.602443268
hsa-mir-151	30.40933518	32.29093225	-1.8003	0.287114879	2.24E-21	3.482926432
hsa-mir-150	30.47592494	24.6518619	5.9053	59.93388544	7.01E-21	0.016685052
hsa-mir-486	28.73173943	31.56628224	-2.7533	0.148311256	4.31E-20	6.74257657
hsa-mir-197	30.22004439	32.22121169	-1.9199	0.264272828	5.34E-18	3.783968293
hsa-mir-140	30.80686384	28.81119314	2.077	4.219289268	2.87E-17	0.237006741
hsa-mir-320	32.3865371	33.86695527	-1.3991	0.379165604	1.63E-16	2.637370031
hsa-mir-374	34.51049488	32.67750663	1.9143	3.76930882	8.01E-15	0.265300629
hsa-mir-019a	32.11311761	29.96710463	2.2273	4.682568177	2.86E-12	0.213558022
hsa-mir-019b	29.4539469	27.85115537	1.6841	3.213398715	7.12E-12	0.311196988
hsa-mir-126	28.07632355	29.10031739	-0.9427	0.520258307	2.61E-11	1.922122121
hsa-mir-016	27.74576263	26.5689361	1.2581	2.391805375	1.35E-10	0.418094219
hsa-mir-532	36.36702118	34.20639555	2.2419	4.730196117	6.15E-10	0.211407725
hsa-mir-092	29.75216725	30.55396618	-0.7205	0.606887075	1.17E-09	1.647753003
hsa-mir-199a*	33.58726049	35.4571252	-1.7886	0.289452796	3.21E-09	3.454794744
hsa-let-7g	33.60687451	31.98889118	1.6993	3.247433539	4.99E-09	0.307935478
hsa-mir-032	29.54416735	31.80526606	-2.1798	0.220706343	8.52E-09	4.530907381
hsa-mir-345	36.51374094	34.71916814	1.8759	3.670305114	1.26E-08	0.272456913
hsa-mir-103	33.43298386	34.64525506	-1.131	0.456599125	1.99E-08	2.190104942
hsa-mir-021	34.3396759	32.34668429	2.0743	4.211400264	4.23E-08	0.237450714
hsa-mir-183	32.45422284	35.11564953	-2.5801	0.167229352	7.17E-08	5.979811469
hsa-mir-142-5p	33.80914806	32.4729949	1.4174	2.671037073	9.83E-08	0.374386417
hsa-mir-017-5p	31.31194325	32.41959375	-1.0264	0.490933663	1.26E-07	2.03693508
hsa-mir-106b	33.74281576	32.50763722	1.3165	2.490611501	1.28E-07	0.401507822
hsa-mir-342	30.04861659	29.34785373	0.782	1.719512972	3.06E-07	0.581560021

**Figure 10 – Table V**

hsa-mir-015a	36.02643541	34.41777394	1.6899	3.226343396	3.25E-07	0.309948408
hsa-mir-106a	32.27341998	33.310393	-0.9557	0.515591362	4.92E-07	1.939520467
hsa-mir-030a-5p	32.84903206	32.11457514	0.8157	1.760151976	4.98E-07	0.56813276
hsa-mir-181d	36.91248884	35.10117465	1.8926	3.713037794	5.30E-07	0.269321255
hsa-mir-574	29.77877718	31.99443544	-2.1344	0.227762162	3.05E-06	4.390544899
hsa-mir-020a	29.10969835	28.36665692	0.8243	1.770675693	3.74E-06	0.564756157
hsa-mir-133b	35.04172861	36.86655569	-1.7435	0.298644281	4.05E-06	3.348465259
hsa-let-7b	32.39289143	33.23120741	-0.757	0.591725511	4.25E-06	1.689972769
hsa-mir-026b	32.29385324	31.16910598	1.206	2.30697121	5.44E-06	0.433468782
hsa-mir-027b	34.97319163	37.12771071	-2.0732	0.23763183	1.20999E-05	4.208190455
hsa-mir-223	21.63665394	21.16179467	0.5561	1.470289246	2.99379E-05	0.680138281
hsa-mir-195	33.54064118	32.45677802	1.1652	2.242643031	0.000055077	0.44590244
hsa-mir-024	27.78702461	28.36258525	-0.4943	0.709906043	0.000137267	1.408637114
hsa-mir-030d	33.8831652	32.98623929	0.9782	1.970005968	0.000154741	0.507612675
hsa-mir-015b	31.44832671	32.07442065	-0.5448	0.685486424	0.000371404	1.458818095
hsa-mir-096	31.46980261	32.73422363	-1.1831	0.44040416	0.000386324	2.270641588
hsa-mir-191	26.63890682	27.27556098	-0.7068	0.612677595	0.00047708	1.632179808
hsa-mir-425-3p	34.77417378	35.82356186	-0.9681	0.511178832	0.00084906	1.95626254
hsa-mir-020b	34.97815304	33.84422814	1.2152	2.321729667	0.000852341	0.430713366
hsa-mir-643	35.6734501	37.16658316	-1.4118	0.375842469	0.00130314	2.660689207
hsa-mir-126*	31.16681945	32.1345669	-0.8865	0.54092482	0.001314585	1.84868574
hsa-mir-423	33.93739253	34.82278949	-0.8041	0.57271925	0.001362239	1.746056205
hsa-mir-425-5p	33.23637712	33.88098071	-0.5633	0.676752398	0.00179719	1.477645299
hsa-mir-026a	27.61150875	27.32677761	0.366	1.28877463	0.003310506	0.775930854
hsa-mir-302b	33.23113157	34.33434284	-1.0219	0.492467356	0.003731969	2.030591447
hsa-mir-484	26.55751618	28.13046808	-1.4917	0.355593289	0.009046242	2.812201558
hsa-mir-125a	30.94104939	31.63410639	-0.6118	0.654379746	0.02785181	1.528164657
hsa-let-7a	34.16573733	34.66154112	-0.4145	0.750279477	0.031855987	1.332836671
hsa-mir-628	33.65892745	34.23451988	-0.4943	0.709906043	0.033363855	1.408637114
hsa-mir-182*	34.51246341	35.19580641	-0.6021	0.65879431	0.04206113	1.517924464
hsa-mir-093	29.92991163	30.33080725	-0.3196	0.801292012	0.043597802	1.247984486
hsa-mir-376a	35.49479343	36.2218518	-0.6458	0.639138279	0.048003434	1.564606648
hsa-mir-196b	33.89190492	34.56216867	-0.589	0.664803554	0.052904716	1.504203751
hsa-mir-025	36.27231106	35.85259422	0.501	1.41519416	0.069149461	0.706616822
hsa-mir-027a	33.33901471	32.99540369	0.4249	1.342479446	0.101287776	0.744890362
hsa-mir-146a	27.051654	26.95885476	0.1741	1.12826034	0.241458101	0.886320262
hsa-mir-340	35.71472961	35.53800265	0.258	1.195819797	0.297093531	0.8362464
hsa-mir-030b	30.55810765	30.72009382	-0.0807	0.945598728	0.389875101	1.057531033
hsa-mir-186	31.31177086	31.45367741	-0.06062	0.958851963	0.659198081	1.042913858
hsa-mir-331	31.20308243	31.25979306	0.02458	1.017183525	0.843135202	0.983106761
hsa-mir-030c	30.93719829	31.01586004	0.002625	1.001821168	0.97816881	0.998182143

Figure 10 – Table V (cont)

Table VI

detector_name	Average Normalized Expression PBMC	PBMC Standard deviation	detector_name	Average Normalized Expression Plasma	Plasma Standard deviation
hsa-mir-223	2143.797514	499.6723532	hsa-mir-223	1589.265353	653.1441
hsa-mir-150	241.3339986	94.6316509	hsa-mir-484	50.93154102	22.91411
hsa-mir-146b	57.51515588	21.17347322	hsa-mir-191	46.44422571	14.98219
hsa-mir-016	54.79023342	32.92864759	hsa-mir-146a	39.5631478	19.02017
hsa-mir-484	40.62828652	18.89513879	hsa-mir-016	25.45043823	13.32435
hsa-mir-146a	39.66536453	13.01422255	hsa-mir-026a	25.2068328	9.956682
hsa-mir-191	32.44104295	15.62493155	hsa-mir-222	24.51626706	12.42177
hsa-mir-026a	30.05789737	8.928309461	hsa-mir-024	22.79169634	10.53596
hsa-mir-019b	21.72111133	7.496587814	hsa-mir-126	18.20923598	8.04666
hsa-mir-020a	15.03972998	5.117918017	hsa-mir-032	15.32305403	32.61654
hsa-mir-024	14.49037987	2.924884427	hsa-mir-486	12.89548349	11.97428
hsa-mir-142-3p	11.61802275	7.535807778	hsa-mir-020a	10.12067658	5.597534
hsa-mir-140	11.15286241	3.933184767	hsa-mir-019b	9.158209689	6.76422
hsa-mir-126	9.209558485	3.825687483	hsa-mir-150	8.165508668	25.08223
hsa-mir-342	7.803100549	3.388772813	hsa-mir-574	5.939301367	3.722014
hsa-mir-155	6.584151232	4.579272166	hsa-mir-092	5.700980896	3.157407
hsa-mir-222	5.715723219	2.105344287	hsa-mir-093	5.436799962	2.417329
hsa-mir-019a	5.53562668	2.957936571	hsa-mir-342	5.114543492	3.297305
hsa-mir-093	3.756090874	1.000518353	hsa-mir-197	4.9808586	4.507
hsa-mir-092	3.215354275	0.85390195	hsa-mir-328	4.707858075	3.592588
hsa-mir-486	3.096228853	7.676887526	hsa-mir-096	3.674085488	5.965292
hsa-mir-030b	2.865611791	0.76538861	hsa-mir-151	3.452172299	1.08377
hsa-mir-574	2.458205492	1.894539696	hsa-mir-146b	3.272000368	2.101495
hsa-mir-030c	2.335820956	0.663949738	hsa-mir-140	3.196516876	1.71678
hsa-mir-026b	2.129402092	0.705755408	hsa-mir-030b	3.134519745	1.142809
hsa-mir-331	1.985929854	0.609153141	hsa-mir-125a	3.080410769	1.975409
hsa-mir-125a	1.808127477	1.087896802	hsa-mir-126*	3.023376204	2.068206
hsa-mir-186	1.720577641	0.419612713	hsa-mir-183	2.854264163	6.494415
hsa-mir-032	1.716467503	1.338128537	hsa-mir-030c	2.412532336	0.863587
hsa-mir-029a	1.323099465	0.533548819	hsa-mir-017-5p	2.13024101	0.998316
hsa-mir-126*	1.269368187	0.759421276	hsa-mir-331	2.087433557	0.81255
hsa-let-7g	1.199246639	0.349725473	hsa-mir-186	2.022411766	0.916917
hsa-mir-021	1.161213483	0.674584117	hsa-mir-015b	1.808314178	0.81048
hsa-mir-197	1.149407136	0.647186919	hsa-mir-019a	1.697172987	1.453897

Figure 11 – Table VI

hsa-mir-015b	1.14628446	0.432407565	hsa-mir-302b	1.339313335	2.387044
hsa-mir-030a-5p	1.094683149	0.306485271	hsa-mir-026b	1.292369054	0.824158
hsa-mir-195	1.017673959	0.797387672	hsa-mir-106a	1.186157531	1.293289
hsa-mir-151	1.007960478	0.397967603	hsa-let-7b	0.97666637	0.625536
hsa-mir-142-5p	0.962665368	0.492490733	hsa-mir-320	0.911092198	0.420503
hsa-mir-017-5p	0.941159371	0.360846484	hsa-mir-155	0.808867743	0.52866
hsa-mir-106b	0.910164376	0.403715009	hsa-mir-030a-5p	0.727538734	0.549257
hsa-mir-096	0.875989336	0.559638262	hsa-mir-628	0.713396354	1.263219
hsa-mir-374	0.764917256	0.274388929	hsa-mir-027a	0.641903058	0.448917
hsa-mir-328	0.720096352	0.412158586	hsa-mir-142-3p	0.627217683	0.607184
hsa-mir-030d	0.662654685	0.22361572	hsa-mir-195	0.564628001	0.392046
hsa-mir-027a	0.622821419	0.251240619	hsa-mir-425-5p	0.554428361	0.275865
hsa-mir-106a	0.542577626	0.312616405	hsa-let-7g	0.515997707	0.337364
hsa-let-7b	0.534079686	0.25068168	hsa-mir-021	0.51383279	0.491008
hsa-mir-020b	0.410435233	0.262371622	hsa-mir-199a*	0.503454664	0.327551
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hsa-mir-628	0.323074686	0.328272174	hsa-mir-106b	0.455236625	0.295268
hsa-mir-302b	0.31107497	0.233922375	hsa-mir-182*	0.450866408	0.779637
hsa-mir-532	0.30283697	0.175299492	hsa-mir-196b	0.424682779	0.283318
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hsa-mir-015a	0.263528933	0.156658681	hsa-mir-030d	0.360317322	0.195419
hsa-mir-183	0.223615225	0.225419033	hsa-mir-423	0.355300524	0.267033
hsa-mir-345	0.222589939	0.14617654	hsa-let-7a	0.316043353	0.197157
hsa-mir-423	0.213547231	0.136842846	hsa-mir-027b	0.252490316	0.182518
hsa-mir-103	0.205412359	0.093491856	hsa-mir-374	0.251770179	0.197212
hsa-let-7a	0.198065472	0.082808812	hsa-mir-020b	0.227198431	0.167979
hsa-mir-181d	0.170055022	0.109234879	hsa-mir-133b	0.201227753	0.178372
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hsa-mir-340	0.128492001	0.08303611	hsa-mir-376a	0.166829158	0.161986
hsa-mir-425-3p	0.127886265	0.097067479	hsa-mir-340	0.113550436	0.088995
hsa-mir-199a*	0.127527328	0.079775882	hsa-mir-015a	0.110336695	0.095621
hsa-mir-376a	0.124903907	0.160137603	hsa-mir-181d	0.093074354	0.212993
hsa-mir-643	0.116164417	0.195641411	hsa-mir-532	0.086633253	0.064244
hsa-mir-025	0.11319355	0.079644761	hsa-mir-025	0.072911126	0.043857
hsa-mir-133b	0.100108157	0.121551814	hsa-mir-345	0.071420142	0.054899
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Figure 11 – Table VI (cont)

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